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(54) Title: TREATMENT AND PROPHYLAXIS OF DISEASES CAUSED BY PARASITES, OR BACTERIA

(57) Abstract

Aromatic compounds, or prodrugs thereof, which contain an alkylating site and which are capable of alkylating the thiol group in N-acetyl-L-cysteine, in particular bis-aromatic α,β-unsaturated ketones, are used for the preparation of pharmaceutical compositions or medicated feed, food or drinking water for the treatment or prophylaxis of diseases caused by microorganisms or parasites, in particular protozoa such as *Leishmania, Trypanosoma, Toxoplasma, Plasmodium, Pneumocystis, Babesia* and *Theileria*, intestinal protozoa such as *Trichomonas* and *Giardia; Coccidia* such as *Eimeria, Isospora, Cryptosporidium; Cappilaria, Microsporidium, Sarcocystis, Trichodina, Trichodinella, Dacthylogurus, Pseudodacthylogurus, Acantocephalus, Ichthylophtherius, Botrecephalus*; and intracellular bacteria, in particular *Mycobacterium, Legionella* species, *Listeria* and *Salmonella*. Preferred compounds have the formula (II): X_m-Ph-C(O)-CH=CH-Ph-Y_n, wherein each phenyl group (Ph) may be mono- or polysubstituted; X and Y designate AR_H or AZ, wherein A is O, S, NH or N(C_{1-6}alkyl), R_H designates aliphatic hydrocarbyl, and Z is H or a masking group which is decomposed to liberate AH; m is 0, 1 or 2, and n is 0, 1, 2 or 3, whereby, when m is 2, then the two X are the same or different, and when n is 2 or 3, then the two or three Y are the same or different, with the proviso that n and m are not both 0. As examples of such compounds, chalcones, e.g. licochalcone A (obtainable i.a. from batches of Chinese licorice root of Glycyrrhiza species, e.g. *G. uralensis* or *G. inflata*) as well as hydroxy, alk(en)yl, and/or alk(en)yloxy analogues thereof are active *in vitro* and/or *in vivo* against i.a. *L. major* and *P. falciparum*.

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TREATMENT AND PROPHYLAXIS OF DISEASES CAUSED BY PARASITES, OR BACTERIA

The present invention relates to the use of a particular class of aromatic compounds, in particular bis-aromatic α,β-unsaturated ketones, most of which are novel compounds, for the treatment or prophylaxis of a number of serious conditions caused by microorganisms or parasites, in particular protozoa such as *Leishmania, Plasmodia,* and *Coccidia* such as *Eimeria*, and intracellular bacteria, including *Legionella* and *Mycobacteria*. The invention also relates to the novel bis-aromatic α,β-unsaturated ketones and methods of preparing them, as well as to pharmaceutical and antiparasitic compositions. Furthermore, the invention also relates to a method for treatment or prophylaxis of diseases caused by microorganisms or parasites.

Parasitic diseases, among these malaria and leishmaniasis, are, on a world basis, among the most important diseases. The most effective known drugs against the diseases have many side effects for which reason it is not possible to maintain the treatment or prophylaxis of specific diseases for years.

Recently, the development of resistance against the available drugs against particularly malaria and leishmania parasites has been reported.

Especially malaria and leishmaniasis remain serious diseases despite the efforts to control the diseases and reduce their prevalence by vector eradication and drug treatment.

More than 12 million people in the world are inflicted by leishmaniasis. There are more than 400,000 new cases and 100,000 deaths each year but as many as 350 million people are at risk of infection (WHO,1990). The annual incidence of clinical leishmaniasis is estimated to exceed 2,000,000 cases in some 80 countries. It is one of the 7 important tropical diseases included in the TDR program.

Leishmaniasis are characterized by a broad spectrum of clinical manifestations depending on the strain of the parasite and the host immune response. The parasites infect macrophages and multiply inside these cells. The first step in the Leishmania/macrophage interactions is the binding of the parasite to the macrophage followed by uptake of the parasite. Integrity and fluidity of the host cell membrane is essential for this interaction. Certain parasite surface antigens such as membrane glycoprotein (Gp63) and lipophosphoglycan (LPG) as well as a number of macrophage surface receptors are also important in binding and uptake of the parasite by macrophages.

Various species of the protozoan parasite *Leishmania* cause a broad spectrum of

diseases ranging from the cutaneous healing skin lesions caused by *L. major* to a fatal visceral form of the disease called kala azar caused by *L. donovani* (Manson-Bahr, 1987). Leishmaniases are widespread in many parts of the world with highest prevalence in Africa, Asia, and Latin America (WHO, 1989). Recently an increasing number of AIDS patients are becoming infected with *Leishmania* (Brenguer, 1989; Flegg 1990).

Therapy of patients with leishmaniasis still poses a serious problem. Most of the available antileishmanial drugs exhibit considerable toxicity and there are reports of large scale clinical resistance to the conventional antimonial drugs. No effective, safe, and nontoxic antileishmanial drug is available at present.

There are also reports of large scale clinical drug resistance in visceral leishmaniasis. (TDR News No. 34, 1990)

Malaria, another parasitic disease, is also a serious health problem. Human malaria is caused by four species of the protozoan genus, *Plasmodium*. The species *Plasmodium falciparum* is the most dangerous, causing acute severe infections that are often fatal, especially in young children and immigrants entering endemic areas. The life cycle of *P. falciparum* includes different stages; in the first stage, the sporozoite stage, the parasite is brought into the blood stream by the Anopheles mosquito. The sporozoites are carried in the blood stream to the liver where they invade the hepatocytes and develop into merozoites in the course of 5-7 days. Merozoites released from infected cells start a new cycle by invading the erythrocytes. It is the invasion of the erythrocyte which gives rise to the clinical disease. In the erythrocyte, the parasite shows an asexual multiplication which involve a maturation of the parasite through different parasite stages, the ring, the trophozoite and the schizont stage (the stage that undergoes nuclear division). When the schizont-infected erythrocyte bursts, new merozoites are released. Some merozoites, however, differentiate into gametocytes (microgametocytes and macrogametocytes), the sexual form of the parasite. Contrary to the asexual infected erythrocytes, these sexual parasite stages are able to continue the life cycle when the infected cells, the erythrocytes, are ingested by mosquitoes during a blood meal. By fertilization in the mosquito gut, the gametocytes develop into a mobile ookinete stage. The ookinete pass through the epithel and matures into a oocyst. In the oocyst, the new sporozoites develop. These sporozoites are released and move to the salivary gland, and are then ready to be injected into a new host. The parasites are haploid in most of the life cycle as they perform a meiotic cell division shortly after fertilization. The Anopheles mosquito is the primary vector of malaria, but the disease can be seen after blood transfusion, i.v. injection of medicaments and after transfer from an infected mother to the newborn child through the placenta.

Each year, several hundreds of millions of human beings are affected by the parasitic disease malaria. The treatment and prophylaxis of malaria has been difficult because the available drugs exhibit severe side effects, and furthermore, the *Plasmodia* are showing increasing resistance towards the drugs (Ann (WHO) 1990).

Coccidial protozoa such as *Eimeria tenella* are some of the most important parasites causing disease in poultry resulting in significant economic loss. There are problems with resistance development against some of the available anticoccidial drugs used in prophylaxis and treatment of these diseases, for which reason there is a need for development of new anticoccidial drugs.

Also, *Babesia* species cause devastating damage to cattle in many parts of the world, and there is a need for the development of safe, effective and inexpensive drugs to control these diseases.

Thus, there is a great need for effective drugs against parasitic diseases, especially for drugs exhibiting none or only less severe side effects.

According to the present invention, it has been found that a class of aromatic compounds, said class comprising compounds containing an alkylating site, show a remarkable capability of effectively suppressing the growth of parasitic protozoa and intracellular bacteria, which compounds at the same time can be so chosen that they are tolerable to animal cells such as human cells. This valuable selective activity of such alkylating aromatic compounds seems to be based on their capability of interfering with oxygen metabolism in the parasites by destroying their mitochondria, at concentrations at which the compounds, while thus being harmful to the microorganisms, do not affect the mitochondria of the animal cells.

Without being limited to any particular theory, it is believed that the capability of the compounds to alkylate nucleophilic groups in biomolecules, as evidenced by their capability of alkylating the thiol group of N-acetyl-L-cysteine, is of importance for the antimicrobial effect.

In accordance with this, the present invention, in its broadest aspect, relates to the use of an aromatic compound which contains an alkylating site, and which is capable of alkylating the thiol group in N-acetyl-L-cysteine at physiological pH, for the preparation of a pharmaceutical composition or a medicated feed, food or drinking water for the treatment or prophylaxis of a disease caused by a microorganism or a parasite in an animal, including a vertebrate, such as a bird, a fish or a mammal, including a human,

the microorganism or parasite being selected from

parasitic protozoa, in particular tissue and blood protozoa such as *Leishmania, Trypanosoma, Toxoplasma, Plasmodium, Pneumocystis, Babesia* and *Theileria;* intestinal protozoan flagellates such as *Trichomonas* and *Giardia;* intestinal protozoan *Coccidia* such as *Eimeria, Isospora, Cryptosporidium; Cappilaria, Microsporidium, Sarcocystis, Trichodina, Trichodinella, Dacthylogurus, Pseudodactylogurus, Acantocephalus, Ichthyophtherius, Botrecephalus;* and intracellular bacteria, in particular *Mycobacterium, Legionella* species, *Listeria,* and *Salmonella.*

As it will appear from the following, the aromatic compound may in many cases advantageously be used in the form of a prodrug of the aromatic compound, and it will be understood that the present broadest aspect of the invention encompasses the use of such prodrugs. Expressed in another manner, the broadest aspect of the invention relates to a method for the treatment or prophylaxis of a disease caused by a microorganism or a parasite selected from the protozoa and bacteria stated above, the method comprising administering, to an animal in need thereof, an effective amount of an aromatic compound which contains an alkylating site, and which is capable of alkylating the thiol group in N-acetyl-L-cysteine at physiological pH, or a prodrug thereof.

From the description which follows, it will be seen that a large number of aromatic compounds which show the above-mentioned selective effect are compounds which have one or several electron-donating groups such as hydroxy or derivatives thereof substituted on an aromatic ring. It is believed that the above-described selectivity is obtained through such adequate substitution which modifies the alkylating potency. It will also appear from data described herein that important representatives of the compounds in question are compounds which contain an aromatic ring attached to the alkylating site.

As appears from the following, convenient and reproducible *in vitro* tests have been devised to test the selectivity of aromatic N-acetyl-L-cysteine-thiol-alkylating compounds, and based on a large number of tested compounds, it has been found that the above-mentioned aromatic N-acetyl-L-cysteine-thiol-alkylating compounds in which one or several electron-donating groups such as hydroxy or derivatives thereof is/are present on an aromatic ring, are almost consistently capable of showing a useful selectivity, resulting in effective suppression of the growth of pathogenic microorganisms or parasites in concentrations which are well tolerated by animal cells.

The *in vitro* tests involve establishing the inhibition of the multiplication of the

protozoa or bacteria on the one hand and the animal cells on the other hand by determining the inhibition of the uptake of radiolabelled precursors as an indication of the inhibition of the growth of the parasite or the animal cells in the presence of the test compound in the concentration in question (see Example 14 herein and the examples to which it refers).

The tests involve a particularly suitable assay for assessing the tolerability of the aromatic alkylating compounds to animal cells, that is, an assay based on the assessment of the reduction caused by the compound on the thymidine uptake by lymphocytes of the animal in the Lymphocyte Proliferation Assay (LPA) which is the assay described in greater detail in Example 13.

It has also been found that compounds which are found to be promising in the *in vitro* model also cure animals infected with leishmania and malaria parasites, respectively, such as was shown in a suitable model involving intraperitoneal administration of the compounds to mice or hamsters (see Examples 8, 9 and 16).

Furthermore, it has been found that compounds with antileishmanial and antimalarial activity exhibit inhibitory effect on the growth of intracellular bacteria such as *Mycobacteria* which causes tuberculosis in humans, and *Legionella* which causes legionnaires disease in humans (see Examples 17 and 19).

The fact that these compounds exhibit strong antiparasitic activity against several species of two important human protozoan parasites, *Plasmodium* and *Leishmania*, and against *Eimeria tenella*, the most important parasite in poultry (see Example 28) makes it justified to presume that these compounds will also be strongly active against important veterinarian protozoan parasites such as *Babesia* in cattle, which is intraerythrocytic similar to the malaria parasite, other *Coccidia* in poultry, and *Pseudodactylogurus* or *Trichodina* in fish.

Furthermore, based on the broad spectrum antimicrobial activity of the compounds (see Examples, 17, 18 and 19), it can be assumed that these compounds have similar activity against other microorganisms such as *Salmonella*, and *Trichinella*, and quite generally against a broad range of microorganisms as defined below, in particular aerobic microorganisms and, among those, in particular microorganisms which are found in tissues and host cells of an infected animal.

While it has been established that the alkylating site may be a carbon-carbon double bond conjugated with a carbonyl group, it is contemplated, based on general chemical considerations, that it may also be a carbon-carbon triple bond conjugated with a carbonyl group, or an epoxy group. It is preferred that the alkylating site is a double or

triple bond (from the point of view of availability of the compound preferably a double bond) conjugated with a carbonyl group. The carbonyl group may be the carbonyl group of an aldehyde or a ketone, or it may be the C=O group of a carboxylic acid group or a derivative thereof such as an ester.

In a preferred class of compounds, the carbonyl group is a ketonic carbonyl group which is further conjugated with an aromatic ring, such as a phenyl group. In this case, the phenyl group may carry electron-donating groups, confer what is discussed above, in particular one or several hydroxy groups or derivatives thereof. In the case of hydroxy groups, these may be masked in order to prevent metabolism, confer the detailed discussion further below. The masking groups are preferably chosen from groups from which the free phenol may be released in the body, either enzymatically or non-enzymatically.

Considering that human lymphocytes are representatives of sensitive animal cells, it is, as a general rule, it is preferred according to the present invention that the aromatic alkylating compound is one which, in a concentration in which it causes less than 50% reduction, preferably less than 40% reduction, and more preferably less than 20% reduction, of the thymidine uptake by human lymphocytes in the Lymphocyte Proliferation Assay using phytohemagglutinin (PHA), meets at least one of the following criteria:

a) the aromatic compound is capable of inhibiting *in vitro* the growth or multiplication of *Leishmania major* promastigotes by at least 80%, as determined by uptake of tritiated thymidine,

b) the aromatic compound is capable of inhibiting *in vitro* the growth or multiplication of *Plasmodium falciparum* by at least 80%, as determined by uptake of tritiated hypoxanthine,

c) the aromatic compound is capable of inhibiting *in vitro* the growth or multiplication of *Eimeria tenella* in chicken fibroblast cell cultures by at least 70% ,as determined by counting the parasites,

d) the aromatic compound is capable of inhibiting *in vitro* the growth or multiplication of *Mycobacterium tuberculosis* or *Legionella pneumophila* by at least 50%, as determined by colony counts.

However, it will be understood that the most important consideration is that the compound is tolerable to the animal in concentrations in which it will control the protozoa or the intracellular bacteria. In particular, preferred compounds to be used

according to the invention are compounds which meets all of the criteria a) to d), because this is an indication of a broad-spectred activity and selectivity.

According to an embodiment of the use according to the present invention, the pharmaceutical composition prepared is a composition for the treatment or prophylaxis of diseases caused by *Leishmania* in humans or dogs, and the aromatic compound used is capable of inhibiting *in vitro* the growth of *Leishmania major* promastigotes by at least 80%, as determined by uptake of tritiated thymidine, in a concentration of the compound in which it causes less than 50% reduction, preferably less than 40% reduction, more preferably less than 20% reduction, of the thymidine uptake by human lymphocytes in the Lymphocyte Proliferation Assay using PHA.

In the following, reasonable selection criteria based upon the behaviour of the compounds in representative tests are stated for compounds to be used for treatment or prophylaxis of a number of diseases, confer the corresponding claims 10-24:

As determined by a representative *in vivo* test, the pharmaceutical composition for the treatment or prophylaxis of diseases caused by *Leishmania* in humans or dogs, is preferably one in which the aromatic compound, or the prodrug, when administered intraperitoneally in the *in vivo* test described in Example 8 herein in a dose of up to 20 mg per kg body weight, especially in a dose of up to 10 mg er kg body weight, once daily for 40 days to female BALB/c mice which have been infected with *L. major* (10^7/mouse), the administration being initiated one week after infection, is capable of preventing increase in lesion size by at least 60%, preferably at least 80%, more preferably at least 90%.

In another embodiment, the pharmaceutical composition is a composition for the treatment or prophylaxis of diseases caused by *Leishmania* in humans or dogs, and the aromatic compound, or the prodrug, when administered intraperitoneally in the *in vivo* test described in Example 9 herein in a dose of up to 20 mg per kg body weight, preferably in a dose of up to 10 mg per kg body weight, two times daily for 7 days to male Syrian golden hamsters which have been infected with *L. donovani* promastigotes (2 x 10^7/hamster), the administration being initiated one day after infection, is capable of reducing the parasite load in the liver of the hamsters by at least 60%, preferably by at least 80%, and more preferably by at least 90%.

In yet another embodiment, the pharmaceutical composition is a composition for the treatment or prophylaxis of malaria caused by *Plasmodium spp.* in humans, and the aromatic compound is capable of inhibiting *in vitro* the growth of *Plasmodium falciparum* by at least 80%, as measured by uptake of tritiated hypoxanthine, in a concentration of the compound in which it causes less than 50% reduction, preferably 40%

reduction, more preferably 20% reduction, of the thymidine uptake by human lymphocytes, as measured by the Lymphocyte Proliferation Assay using PHA.

In yet a further embodiment, the pharmaceutical composition is a composition for the treatment or prophylaxis of diseases caused by *Plasmodium spp.* in humans, and the aromatic compound, when administered intraperitoneally in the *in vivo* test described in Example 16 herein in a dose of up to 20 mg per kg body weight two times daily for 6 days to female BALB/c mice which have been infected with malaria *P. yoelii* (2×10^5/mouse), the administration being initiated one day after infection, is able to prevent increase in the parasitemia during the administration period. In particular, the aromatic compound, or the prodrug, when administered intraperitoneally in the *in vivo* test described in Example 16 herein in a dose of up to 20 mg per kg body weight two times daily for 10 days to 8 weeks old female BALB/c mice which have been infected with malaria *P. yoelii* (2×10^5/mouse), the administration being initiated one day after infection, is capable of clearing the parasite from the mice within at the most 23 days.

Especially, the aromatic compound, or the prodrug, when administered intraperitoneally in the *in vivo* test described in Example 16 herein in a dose of up to 20 mg per kg body weight two times daily for 8 days to 8 weeks old female BALB/c mice which have been infected with malaria *P. yoelii* strain YM (1×10^6/mouse), the administration being initiated one day after infection, is capable of clearing the parasite from the mice within at the most 21 days, preferably within at the most 17 days.

It is also preferred that the aromatic compound, or the prodrug, when administered intraperitoneally in the *in vivo* test described in Example 16 herein in a dose of 5 mg per kg body weight two times daily for 10 days to 8 weeks old female BALB/c mice which have been infected with malaria *P. yoelii* (2×10^5/mouse), the administration being initiated one day after infection, is capable of clearing the parasite from the mice within at the most 23 days. In particular, the aromatic compound, or the prodrug, when administered intraperitoneally in the *in vivo* test described in Example 16 herein in a dose of 5 mg per kg body weight two times daily for 8 days to 8 weeks old female BALB/c mice which have been infected with malaria *P. yoelii* strain YM (1×10^6/mouse), the administration being initiated one day after infection, is capable of clearing the parasite from the mice within at the most 21 days, preferably within at the most 17 days.

In a further embodiment of the invention, use is made of an aromatic compound, or a prodrug thereof, which aromatic compound contains an alkylating site and which aromatic compound is capable of alkylating the thiol group in N-acetyl-L-cysteine at

physiological pH, for the preparation of a pharmaceutical composition or a medicated feed or drinking water for the treatment or propylaxis of diseases caused by *Coccidia* in poultry such as chickens or turkeys, wherein the aromatic compound, or the prodrug, when administered to chickens with the feed in a concentration of up to 400 ppm for at most 28 days in the *in vivo* test described in Example 28 herein, is capable of controlling infection by *Eimeria tenella* in at least 60% of the chickens and preventing pathological alterations in at least 50% of the chickens, the aromatic compound preferably being one which in a concentration of up to 120 ppm for at most 28 days in the *in vivo* test described in Example 28 herein, is capable of controlling infection by *Eimeria tenella* in at least 60% of the chickens and preventing pathological alterations in at least 65% of the chickens.

In a further embodiment, the pharmaceutical composition is a composition for the treatment or prophylaxis of diseases caused by intracellular bacteria such as *Mycobacteria* in humans or animals such as cattle, and the aromatic compound is one which is capable of inhibiting the growth and multiplication of *Mycobacteria tuberculosis or Legionella pneumophila in vitro* in the test described in Example 17 herein at a mean MIC of 10 µg per ml, and, in the same concentration, causes less than 50% reduction of the thymidine uptake of human lymphocytes as measured by The Lympocyte Proliferation Assay.

The aromatic compound is preferably one which contains an aromatic ring attached to the alkylating site. As indicated above, the compound in particular one which has electron-donating groups attached to an aromatic ring.

In the aromatic compound, the alkylating site is typically a double or triple bond conjugated with a carbonyl group which carbonyl group optionally is further conjugated with an aromatic ring such as a phenyl group, the aromatic ring attached to the alkylating site preferably containing at least one electron-donating group such as an oxygen, nitrogen or sulphur function such as hydroxy, alkoxy (e.g. methoxy), amino, alkylamino, dialkylamino, mercapto, or alkylthio. It is preferred that the electron-donating group(s) is/are attached to the aromatic ring in a position next to and/or most remote relative to the position through which the aromatic ring is attached to the alkylating site.

Particularly important diseases to be treated or prevented by means of the composition prepared according to the invention are human leishmaniasis caused by *Leishmania donovani, L. infantum, L. aethiopica, L. major, L. tropica, L. mexicana complex*, or *L. braziliensis complex* or human malaria caused by *Plasmodium falciparum, P. ovale, P. vivax*, or *P. malariae*, as well as parasitic diseases in livestock, such as *Babesia* in cattle, or a parasitic disease in birds, such as a disease caused by

Coccidia such as *Eimeria tenella* in poultry such as chicken or turkey, or a parasitic disease in fish, such as *Pseudodactylogurus* or *Trichodina*.

The important human malaria parasites with which hundreds of millions of humans are infected, are *Plasmodium falciparum*, *P. ovale*, *P. vivax*, and *P. malariae*. In particular, *Plasmodium falciparum* is the most important human parasite and the number one parasite killer of mankind. The malaria parasites show widespread resistance against almost all available antimalarial drugs. For this reason, the fact that a new class of antimalarial drugs, chemically unrelated to the known antimalarial drugs has been provided, is a feature of the invention which is of great importance. Another important aspect of the invention is that malaria parasites resistant against Chloroquine, the most commonly used antimalarial drug, show very high degree susceptibility to the compounds described herein (Example 15).

Another important aspect of the invention is the antileishmanial activity of the compounds defined above. Visceral leishmaniasis, caused by *Leishmania donovani* or *L. infantum*, inflicts several million people in the world, and this disease recently appears to be a major problem for AIDS patients coming in contact with Leishmania parasites, combined with large scale clinical resistance in endemic areas such as India (which is announced "alarming" by the World Health Organization). Other major diseases are diseases caused by other species of Leishmania, such as *L. aethiopica*, *L. major*, *L. tropica*, *L. mexicana complex*, and *L. braziliensis complex*. Some of these species cause severe disfiguring and morbidity in millions of humans in Central and South America and many parts of Africa.

In one preferred aspect, the invention relates to the use of an aromatic compound which is a bis-aromatic α,β-unsaturated ketone of the general formula I

$$X_m\text{-}Ar^1\text{-}CO\text{-}W\text{-}Ar^2\text{-}Y_n \qquad \text{I}$$

wherein

W is either -CR=CR- or -C≡C-, wherein each R independently of the other R designates hydrogen, C_{1-3} alkyl, or halogen,

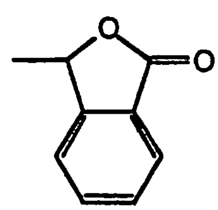
Ar^1 and Ar^2 are the same or different and each designate an aromate selected from phenyl and 5- or 6-membered unsaturated heterocyclic rings containing one, two or three heteroatoms selected from oxygen, sulfur, and nitrogen, such as furanyl, thiophenyl, pyrrolyl, imidazolyl, isoxazolyl, oxazolyl, thiazolyl, pyrazolyl, pyridinyl, or pyrimidinyl, which aromate may be substituted with one or more substituents selected from

halogen; nitro; nitroso; and C_{1-12}, preferably C_{1-6}, straight or branched aliphatic hydrocarbyl which may be saturated or may contain one or more unsaturated bonds selected from double bonds and triple bonds, which hydrocarbyl may be substituted with one or more substituents selected from hydroxy, halogen, amino, and amino which is optionally alkylated with one or two C_{1-6} alkyl groups;

Y and X are the same or different and each designate a group AR_H or a group AZ, wherein A is -O-, -S-, -NH-, or -N(C_{1-6} alkyl)-, R_H designates C_{1-6} straight or branched aliphatic hydrocarbyl which may be saturated or may contain one or more unsaturated bonds selected from double bonds and triple bonds, and Z designates H or (when the compound is a prodrug) a masking group which is readily decomposed under conditions prevailing in the animal body to liberate a group AH, in which A is as defined above; m designates 0, 1 or 2, and n designates 0, 1, 2 or 3, whereby, when m is 2, then the two groups X are the same or different, and when n is 2 or 3, then the two or three groups Y are the same or different, with the proviso that not both of n and m are 0.

When Z designates a masking group, it may typically be selected from the below groups (A)-(E)

-CO-R′	(A)
-CON(CH_3)$_2$	(B)
-CR*R**-O-R″	(C)
-CR*R**-O-CO-R‴	(D)



(E)

wherein R* and R** each independently designate hydrogen or C_{1-3} alkyl, R′, R″ and R‴ each designate C_{1-6} alkyl or is an aromate Ar^1 or Ar^2 as defined above.

Preferred compounds of the general formula I are those, wherein Ar^1 or Ar^2 independently are phenyl or an aromatic 5- or 6-menbered heterocyclic ring containing one, two or three heteroatoms selected from oxygen, nitrogen or sulphur, n is 0, 1, 2, or 3, m is 0, 1 or 2, at least one of the groups X is in a position in Ar^1 most remote rela-

tive to and/or next to the position through which Ar^1 is bound to the carbonyl group, and at least one of the groups Y is in a position in Ar^2 most remote relative to and/or next to the position through which Ar^2 is bound to W.

It is further preferred that A designates O, and Z designates pivaloyl, pivaloyloxymethyl or N,N-dimethylcarbamoyl.

In particular, the bis-aromatic α,β-unsaturated ketones act by selectively destroying the cells of the microorganisms or cells of multicellular parasites; as will appear from the below discussion and the examples herein, the bis-aromatic α,β-unsaturated ketones in appropriate concentration ranges will selectively kill the microorganisms or the multicellular parasites by destroying the cells of the microorganisms or cells of the multicellular parasites while showing a high degree of tolerance for the host cells which are subjected to exposure to the compounds.

As indicated above, it is contemplated (as described in detail in the following description of mechanism) that the mechanism of action is via interference of the O_2-metabolism of the microorganism or parasite in question in that the bis-aromatic α,β-unsaturated ketone inhibits or interferes with the O_2-metabolism of the mitochondria (where applicable) of the microorganism such as the parasite or the O_2-metabolism of the bacteria itself. At the same time, the mitochondria of humans have been found to be able to tolerate the compounds in question in the same concentrations which will inhibit or kill the microorganism or the multicellular parasite. It is this remarkable selectivity of certain classes of bis-aromatic α,β-unsaturated ketones which constitutes the basis of this aspect of the present invention.

Many of the bis-aromatic α,β-unsaturated ketones of the general formula I are novel, and the invention also relates to all such novel bis-aromatic α,β-unsaturated ketones. In the following, some preferred classes of the novel bis-aromatic α,β-unsaturated ketones are defined, and preferred individual compounds among these are discussed specifically.

Because the bis-aromatic α,β-unsaturated ketones used according to the invention have been found to be well tolerated by animal cells, including human cells, such as will be explained in detail in the following, and because these properties are contemplated to be possessed by the broader range of aromatic compounds defined above, the invention opens up the possibility of controlling parasitic diseases not only by administration to the animals, including humans, as therapy or prophylaxis, but also by killing the parasite in its vector by spraying or otherwise applying an aromatic compound of the type defined above, such as a bis-aromatic α,β-unsaturated ketone, in the infected areas so that the vector will take up the compound, whereby the parasite will

be subjected to the compound. Thus, one aspect of the invention relates to a method for controlling transmission of parasitic diseases caused by parasites which have part of their life cycles in a vector, comprising applying an aromatic compound as defined above, such as a bis-aromatic α,β-ketone of the general formula I, to a locus which is a habitat of the vector so as to eradicate the parasites. The parasites will, in this case, in particular be *Leishmania*, *Plasmodium*, or *Trypanosoma*, and the eradication of the parasite will, depending on the vector's tolerance to the compound, take place with or without concomitant eradication of the vector.

When W in the general formula I is -CR=CR-, it may be either cis or trans configured. It is preferred that it is trans configured. It is often preferred that both groups R are hydrogen, but it is contemplated that also bis-aromatic α,β-unsaturated ketones in which one of or both groups R is/are e.g. methyl or ethyl are of great value with respect to the relevant activity and selectivity/tolerability.

With respect to the position of X and/or Y in its/their respective aromate(s), it is highly preferred, and indeed, in number of cases seems to be a condition for a high biological or therapeutic activity against the microorganism in question combined with a high tolerability by animal cells, that at least one of X and Y which is different from hydrogen is positioned in the aromate in a position most remote relative to and/or next to the position through which the aromate is bound to the α,β-unsaturated ketone group. Examples of preferred combinations in this regard are the cases where

- Ar^1 is phenyl or an aromatic heterocyclic ring containing one, two or three heteroatoms, m is 0, 1 or 2, and X is in a position in Ar^1 most remote relative to and/or next to the position through which Ar^1 is bound to the carbonyl group;

- Ar^2 is phenyl or an aromatic heterocyclic ring containing one, two or three heteroatoms, n is 1 , 2 or 3, and each Y is in the a position in Ar^2 most remote relative to and/or next to the position through which Ar^2 is bound to W; or

- Ar^1 and Ar^2 are selected from phenyl and an aromatic heterocyclic ring containing one, two or three heteroatoms, m and n are each 1, 2 or 3, each X is in an position most remote relative to and/or next to the position through which Ar^1 is bound to the carbonyl group, and each Y is in a position most remote relative to and/or next to W.

The aromate is suitably phenyl such as illustrated in most of the examples herein, but it is reasonable to contemplate that any of the aromate types mentioned above can be

the Ar^1 or Ar^2 of the bis-aromatic α,β-unsaturated ketone, considering that such aromatic rings will affect the electron density in the unsaturated ketone similarly to the two phenyl rings, and that such aromates will also give possibilities for charge transfer complexes and lipophilic interactions with the target molecule, such as do the two phenyl rings.

Apart from the important substitution with X and/or Y as explained herein, the aromate may carry other substituents which either will not to any substantial extent detract from the useful effect and selectivity of the bis-aromatic α,β-unsaturated ketones, or will enhance these properties or relevant properties related to the use and utility of the bis-aromatic α,β-unsaturated ketones, e.g., their solubility (such as when the bis-aromatic α,β-unsaturated ketones carry a nitrogen-containing basic group or a carboxyl group which can form water-soluble salts with pharmaceutically acceptable counter ions).

Among the bis-aromatic α,β-unsaturated ketones of the general formula I, the preferred ones are generally those in which A is O, mainly because of their excellent properties with respect to activity and selectivity/tolerability, such as will appear from the results reported herein. However, it is well known that the oxygen atom in the form of oxy in many biologically active compounds may, with greater or lesser retention of, and indeed in certain cases with enhancement of, the biological activity, be replaced with bioisosteric groups, such as -S-, -NH-, and -N(C_{1-6} alkyl)- as mentioned above.

As appears from the discussion herein and the results reported herein, the presence of a particular substituent X or Y or of particular substituents X and Y, preferably in specific positions in the aromate, in particular in the position in the aromate which is remote relative to and/or next to the position of attachment of the aromate, seems to be important to the effect and selectivity of the bis-aromatic α,β-unsaturated ketones. Based upon the above-mentioned general preference for substituents X and Y which contain -O- (but taking into consideration that the oxygen atom could be replaced with the a bioisosteric group), this substituent could be called "an oxy-functional substituent". While it is presumed that the activity of the oxy-functional substituent is related to the substituent in the "free" form, that is, to hydroxy when A is -O-, to thiolo when A is -S-, and to amino or monoalkylamino when A is -NH- or -N(C_{1-6}-alkyl)-, very interesting results obtained with bis-aromatic α,β-unsaturated ketones of the formula I where X or Y is alkenyloxy raise the intriguing question whether the active form in theses cases is the alkenyloxy-substituted form, or whether the alkenyloxy group is converted to a hydroxy group , maybe even by the microorganism or parasite itself, before the bis-aromatic α,β-unsaturated ketone exerts it action. As will be understood, this possibility is covered by the definition R_H above, while the definition of Z , when Z is not hydrogen, is adapted to represent "prodrug" forms

which, in accordance with well known principles used in the construction of suitable administration embodiments of chemical compounds containing, e.g., free hydro groups as substituents on aromatic rings, will be decomposed in the animal body to result in the corresponding compound in which Z is hydrogen.

In a preferred embodiment, the bis-aromatic α,β-unsaturated ketone has the general formula II

$$X_m\text{-Ph-C(O)-CH=CH-Ph-}Y_n \qquad \text{II}$$

wherein Ph designates phenyl, and X_m and Y_n are as defined above, and each phenyl group may be substituted with one or more substituents selected from halogen; nitro; nitroso; and C_{1-12}, preferably C_{1-6}, straight or branched aliphatic hydrocarbyl which may be saturated or may contain one or more unsaturated bonds selected from double bonds and triple bonds, which hydrocarbyl may be substituted with one or more substituents selected from hydroxy, halogen, amino, and amino which is optionally alkylated with one or two C_{1-6} alkyl groups.

In these compounds, it is preferred that X and/or Y designates OH or a group OR_H, in which R_H is as defined above, or OZ^*, in which Z^* is a masking group which is readily decomposed under conditions prevailing in the animal body to liberate the group OH, in particular one of the groups (A)-(E) as defined above, preferably pivaloyl, pivaloyloxymethyl or N,N-dimethylcarbonyl.

The substituent or substituents on the phenyl group(s) is/are preferably selected from C_{1-12}, preferably C_{1-6}, straight or branched aliphatic hydrocarbyl which may be saturated or may contain one or more unsaturated bonds selected from double bonds and triple bonds, which hydrocarbyl may be substituted with one or more substituents selected from hydroxy, halogen, amino, and amino which is optionally alkylated with one and two C_{1-6} alkyl groups.

In especially preferred embodiments, the substituent or substituents on the phenyl groups is/are selected from methyl, ethyl, propyl, isopropyl, tert.-butyl, prop-2-enyl, 1,1-dimethylpropyl, 1,1-dimethylprop-2-enyl, 3-methylbutyl, and 3-methylbut-2-enyl.

The host animals to be treated, either to obtain a therapeutic effect, or to obtain a prophylaxis or protection against infection, are primarily vertebrates such as birds, fish and mammals, including humans. It is evident that with respect to some of the microorganisms and multicellular parasites mentioned above, the host to be treated is defined once the microorganism or multicellular parasite is given. Thus, for example, when the microorganism is *Leishmania*, the hosts to be treated are humans or dogs;

when the microorganism is *Theileria*, the animals to be treated are cattle, sheep and goats; when the microorganism is *Eimeria*, the animals to be treated are chickens and turkeys.

Based upon findings as explained in the examples below, it is presumed that the mechanism of action of the bis-aromatic α,β-unsaturated ketones of the general formulae I and II above, and prodrugs thereof, is as follows:

The bis-aromatic α,β-unsaturated ketones severely damage the mitochondria of the parasites. Mitochondria are oval-shaped organelles, typically about 2 μm in length and 0.5 μm in diameter, located intracellulary in all organisms except bacteria. Mitochondria have two membrane systems, an outer membrane and an extensive, highly folded inner membrane, hence there are two compartments in mitochondria: the intermembrane space between the inner membrane and the outer membrane, and the matrix, which is bounded by the inner membrane.

Mitochondria are the organelles involved in the O_2-metabolism of the cell. Oxidative phosphorylation is the process in which ATP is formed as electrons are transferred from NADH or $FADH_2$ to O_2 by a series of electron carriers. This is the major source of ATP in aerobic organisms. Oxidative phosphorylation is carried out by respiratory assemblies located as an integral part of the inner mitochondrial membrane. The outer membrane is quite permeable to most small molecules and ions.

From B. Inoue, K. Inaba, T. Mori, F. Izushi, K. Eto, R. Sakai, M. Ogata and K. Utsumi, *J. Toxicol. Sci.* 7 (1982), 245-254 it is known that echinastin, 4-hydroxychalcone, chalcone, and 3,4'-dihydroxychalcone cause deterioration of respiratory control and oxidative phosphorylation of isolated rat liver mitochondria. The present inventors have found that bis-aromatic α,β-unsaturated ketones and derivatives thereof of the general formula I or II cause deterioration of respiratory control and oxidative phosphorylation of mitochondria of parasites in concentrations that are so small that the mitochondria of the animal cell are not affected.

Due to the interference with the O_2-metabolism of the mitochondria the mitochondria are destroyed and as a consequence the cell to which the mitochondria belong is destroyed.

Thus, the compound known as licochalcone A does not appear to exhibit any toxicity in animal cells even at fairly high concentrations, cf. the data given in Example 14 herein. Thus, licochalcone A is an important potential antiparasitic, in particular, antimalarial and antileishmanial drug. However, as appears from the experiments reported in the examples herein, the surprising effect and selectivity found is not

limited to licochalcone A, but is characteristic of the class of bis-aromatic α,β-unsaturated ketones discussed herein and, for the reasons given above, is believed to apply more broadly to the aromatic compounds defined above.

Leishmania parasites are transferred through bites from sandflies belonging to the genera *Phlebotomus* and *Lutzomyia*. In the gastrointestinal canal of the flies the parasite is transformed from the amastigote phase to the promastigote phase and is propagated. Thereupon the promastigotes migrate to the mouth, especially the salivary glands of the flies and are transferred with the next bite from the fly.

The promastigotes are bound to the macrophage of the infected organism followed by uptake of the parasite into the macrophage where it is transformed to the amastigote phase and multiply inside these cells.

bis-Aromatic α,β-unsaturated ketones as defined herein have been found to have effect on the *Leishmania* parasite in the amastigote phase as well as in the promastigote phase. This means that the compounds in question are both useful in the prophylaxis of leishmaniasis, because of the effect against the promastigotes, and in the treatment of the disease, because of the effect against the amastigotes. Again, this is believed to apply more broadly to the aromatic compounds defined above.

In cultures, promastigotes multiply with exponential rate the first three days, called the log-phase, and for the following three days the promastigotes are still alive but not multiplying any longer (this phase is called the stationary phase), unless they are transported to another medium. In case the promastigotes are transferred to another medium the log-phase will continue for another three days, and then the promastigotes will enter the stationary phase.

If promastigotes are bound to macrophages in the log-phase, the promastigotes will be killed by the macrophage. On the other hand, if the promastigotes are bound to macrophages in the stationary phase, then the promastigotes are able to infect the cells and multiply inside them.

The stationary phase of the promastigotes, the infective form of the parasite, is generally more sensitive to the bis-aromatic α,β-unsaturated ketones than the log phase of the promastigote, which means that the bis-aromatic α,β-unsaturated ketones are able to prevent infection with the *Leishmania* parasite; in accordance with the explanation given above, this is believed to apply more broadly to the aromatic compounds defined above.

Bacteria possess a cell wall and a cytoplasmic membrane, but lack mitochondria. In-

stead, the electron transport and the oxidative phosphorylation, and the latter only in the aerobic bacteria, takes place in the cytoplasmic membrane which then serves the mitochondria-like function in the bacteria.

It is contemplated that the aromatic compounds, such as the bis-aromatic α,β-unsaturated ketones defined herein interfere with the O_2-metabolism of the cytoplasmic membrane corresponding to the interference with the O_2-metabolism of the mitochondria of higher developed organisms, thereby destroying the bacteria.

As mentioned above, important findings on which the present invention is based are not only the remarkable efficiency of the bis-aromatic α,β-unsaturated ketones with respect to destroying the pathogenic microorganisms, but also the high degree of selectivity which they show with respect to the pathogenic microorganisms as contrasted to animal cells, including human cells. Thus, as will appear from the data given in the examples below, bis-aromatic α,β-unsaturated ketones have been found to be substantially harmless to human cells in concentrations at which they effectively control the parasites. This selectivity was surprising. Moreover, as appears from the examples, a still much higher activity against the microorganisms is found when the microorganisms are present in tissue, such as in cells, such as will be the case in the actual therapeutic use. In many cases, a further increase by a factor 10 in the selectivity is seen.

Preliminary experiments (Example 25) involving oral administration of licochalcone A to mice and rats and injection of licochalcone A to mice indicate that in animals such as mammals, the bis-aromatic α,β-unsaturated ketones which possess a free phenolic hydroxy group will be eliminated from the blood stream already after the first passage to through the liver. This is in accordance with what is known about the metabolism of other phenolic compounds. For this reason, an important aspect of the invention is constituted by compounds in which the phenolic hydroxy group or groups or bioisosteric other group or groups AZ are masked, in other words, the so-called prodrugs, that is, compounds which are readily decomposed under conditions prevailing in the animal body to liberate the free groups which are associated with the active forms of the drugs.

The prodrugs used according to the invention are, e.g., compounds of the general formula I or II in which Z is a group which is readily decomposed under conditions prevailing in the animal body to liberate the group AH. As an important example, when A is O such as is the case in important compounds used according to the invention, it is preferred that Z is a group which is readily decomposed under conditions prevailing in the animal body to liberate the group OH.

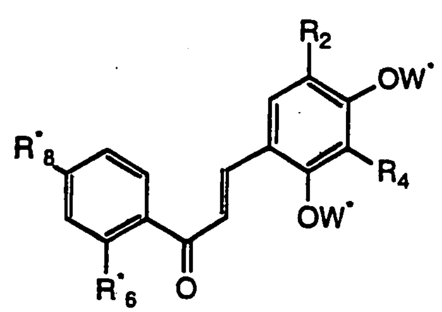
The establishment of prodrug forms suitable in connection with particular substituents in drugs is based upon the fact that certain types of groups will tend to be decomposed in the animal body in accordance with various decomposition pathways. Thus, among the above-mentioned specific prodrug groups (A)-(E), the groups (A), (D), and (E) are groups which will be decomposed by esterases to result in the corresponding free group such as the hydroxy group. The group (B) will be subjected to removal of one of the methyl groups in the liver, and the group thus formed will be relatively readily decomposable in plasma. The oxy-containing groups (C) are groups which are relatively labile under acidic conditions and, as thus, are adapted to be decomposed, e.g., under the conditions under which *Leishmania* amastigotes exist in the human body, that is, in macrophages. Quite generally, the prodrug group Z will be one which prevents the active molecule from being converted, in the liver, to a form which, from a practical point of view, will be inactive and quickly will be eliminated from the animal body, such as the forms where free phenolic OH groups are sulfated in the liver or are coupled to gluconic acid in the liver.

In preferred embodiments, Z is a group selected from the groups (A)-(E) as defined above. Examples of particularly preferred groups Z are pivaloyl, pivaloyloxymethyl and N,N-dimethylcarbamoyl.

The above considerations concerning prodrug derivatives of hydroxy groups in the compounds of the general formula I or II also apply to other hydroxy group-containing aromatic alkylating compounds as defined above.

In the following, valuable and interesting subclasses of the bis-aromatic α,β-unsaturated ketones used according to the invention will be discussed.

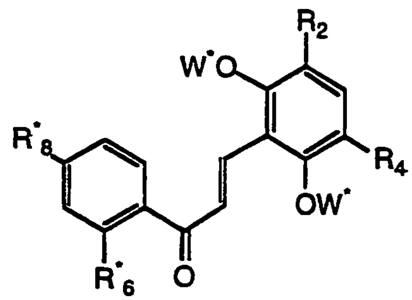
Based upon their generally very interesting selective properties, an interesting class of compounds used according to the invention is constituted by compounds of the general formula III



III

wherein R_2 and R_4 designate R_H as defined above, or H, one of R^*_6 and R^*_8 designate OW^* and the other is H, or both R^*_6 and R^*_8 designate H, and W^* designates H, R_H or a group (A)-(E) as defined above, wherein both R^* and R^{**} designate H.

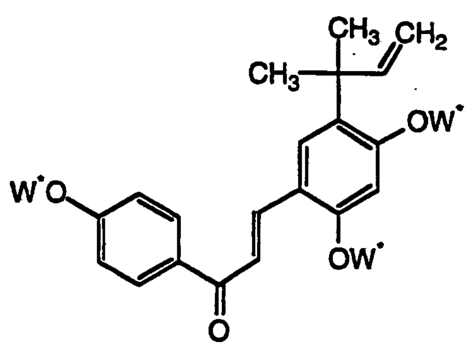
Other interesting bis-aromatic α,β-unsaturated ketones used according to the invention have the general formula IV



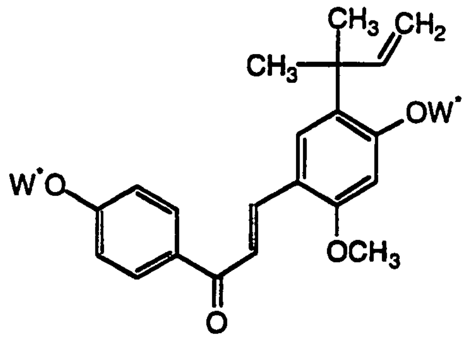
IV

wherein R_2, R_4, R^*_6, R^*_8 and OW^* are as defined above.

Because of the very interesting properties possessed by licochalcone A, confer the examples which follow, very interesting compounds used according to the invention are bis-aromatic α,β-unsaturated ketones in which the two hydroxy groups in licochalcone A are replaced with a group OW^*, in which each W^* independently designates H, R_H or a group (A)-(E) as defined above, wherein both R^* and R^{**} designated H, such as compounds which have the general formula V or VI

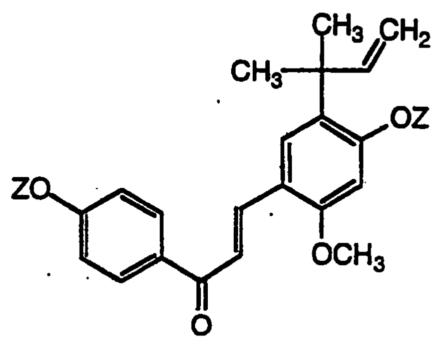


V



VI

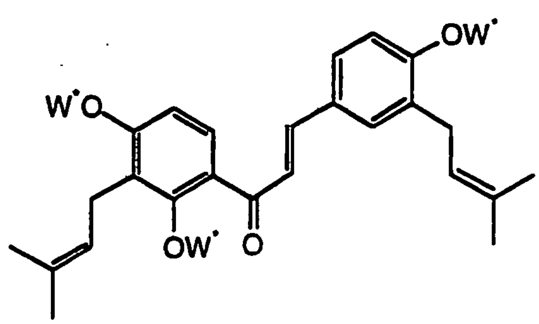
Also, bis-aromatic α,β-unsaturated ketones of the general formula VII



VII

wherein Z is as defined above, are evidently very interesting compounds. In those compounds, it is preferred that Z designates pivaloyl, pivaloyloxymethyl or N,N-dimethylcarbonyl.

Another interesting class of bis-aromatic α,β-unsaturated ketones has the general formula VIII

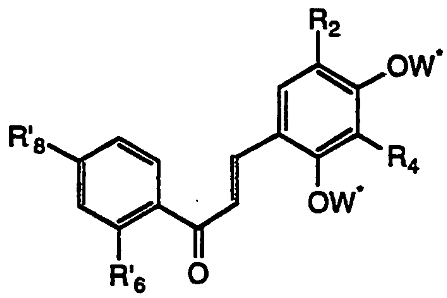


VIII

wherein W* is as defined above.

Many of the bis-aromatic α,β-unsaturated ketones of the general formula I are novel compounds, and the invention also relates to all such novel compounds *per se*.

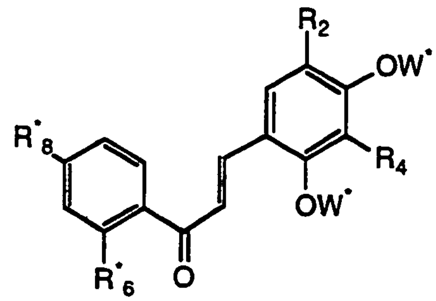
Among the novel compounds of the invention are the bis-aromatic α,β-unsaturated ketones of the general formula IX



IX

wherein one of R'_6 and R'_8 designate $A(W^*)_p$ and the other designates H, or both designate H, A designates S, N or O, whereby, when A designates S or O, then p designates 1, and when A designates N, then p designates 2, with the proviso that when R_2 and R_4 both are H, then at least one W* designates a masking group Z as defined above, andwith the exception of the known compounds licochalcone A, licochalcone C, 3-[4-hydroxy-5-(1,1-dimethylprop-2-enyl)-2-methoxyphenyl]-1-[4-(methoxymethoxy)phenyl]-2-propen-1-one, 3-[4-acetyloxy-5-(1,1-dimethylprop-2-enyl)-2-methoxyphenyl]-1-[4-(methoxymethoxy)phenyl]-2-propen-1-one, 3-[5-(1,1-dimethylprop-2-enyl)-2,4-dimethoxyphenyl]-1-[4-(methoxy)phenyl]-2-propen-1-one, 3-[4-acetyloxy-5-(1,1-dimethylprop-2-enyl)-2-methoxyphenyl]-1-(4-acetyloxyphenyl)-2-prop-1-one, 3-[2-hydroxy-4-methoxy-3-(3-methylbut-2-enyl)phenyl]-1-[4-[(3,7,11-trimethyl-2,6-dodecatri-10-enyl)oxy]phenyl]-2-prop-1-one, and 2,4-dihydroxy-3-methylchalcone.

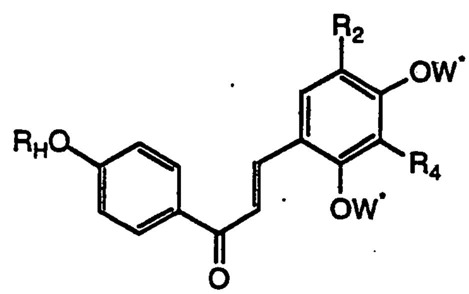
Among such novel compounds of the formula IX, very interesting compound are of the general formula X



X

wherein R_2 and R_4 are as defined in claim 41, one of R^*_8 and R^*_6 designates OW^*, and the other designates H, or both designate H, and W^* is as defined above.

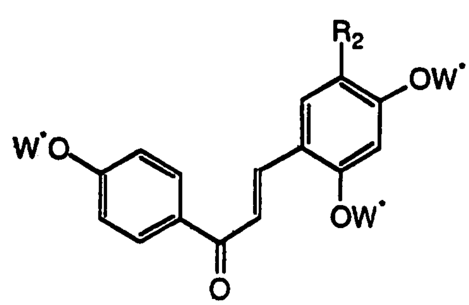
Particularly interesting compounds have the general formula XI



XI

wherein R_2, R_4 and W^* are as defined above. The compounds in which R_2 and/or R_4 designates methyl, ethyl, propyl, isopropyl, tert.-butyl, prop-2-enyl, 1,1-dimethylpropyl, 1,1-dimethylprop-2-enyl, 3-methylbutyl, or 3-methylbut-2-enyl are especially preferred compounds.

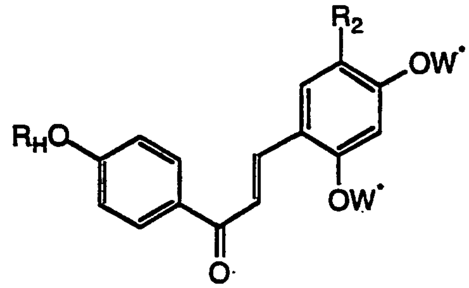
Important novel compounds according to the invention are of the general formula XII



XII

wherein R_2 and W^* are as defined above.

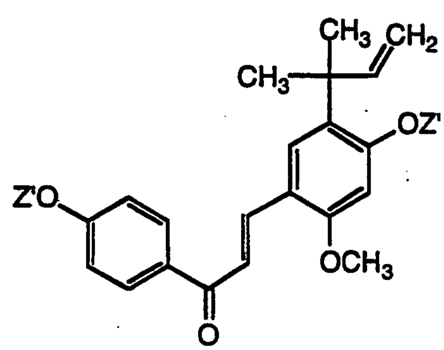
Also, the bis-aromatic α,β-unsaturated ketones of the general formula XIII



XIII

wherein R_H, R_2 and W^* are as defined above are interesting novel compounds. Among these, very interesting compounds are those in which R_2 designates methyl, ethyl, propyl, isopropyl, tert.-butyl, prop-2-enyl, 1,1-dimethylpropyl, 1,1-dimethylprop-2-enyl, 3-methylbutyl, or 3-methylbut-2-enyl.

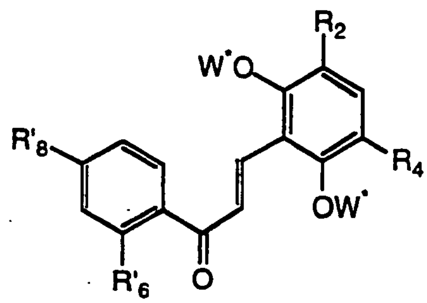
Particularly interesting novel bis-aromatic α,β-unsaturated ketones are prodrugs of Licochalcone A of the general formula XIV



XIV

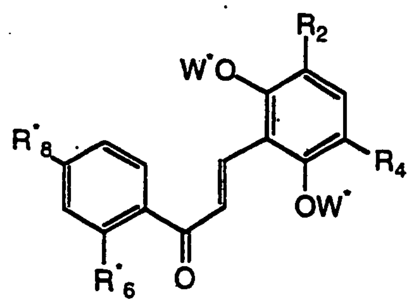
wherein Z′ is one of the groups (A)-(E) as defined above.

Novel bis-aromatic α,β-unsaturated ketones of the general formula XV



XV

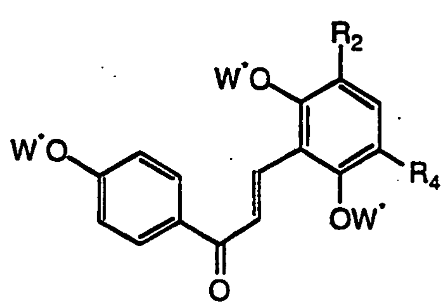
wherein one of R'_6 and R'_8 designates $A(W^*)_p$ and the other designates H, or both designate H, W^* is as defined above, A designate S, N or O, whereby when A designates S or O then p designates 1, and when A designates N then p designates 2, with the exception of 2,6-methoxychalcone and 2-hydroxy-6-methoxychalcone, form a further interesting class of compounds. Of these compounds, an interesting subclass of bis-aromatic α,β-unsaturated ketones have the general formula XVI



XVI

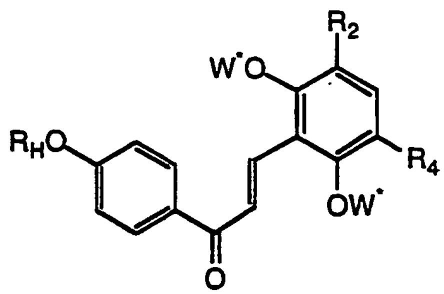
wherein R_2, R_4, R^*_8, R^*_6 and W^* are as defined above.

Among these, interesting novel bis-aromatic α,β-unsaturated ketones have the general formula XVII



XVII

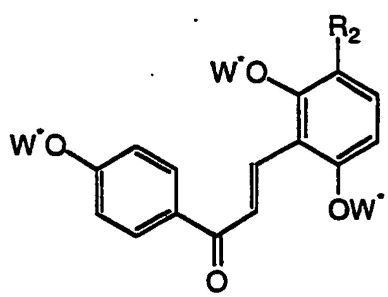
wherein R_2, R_4 and W^* are as defined above, in particular the compounds of the general formula XVIII



XVIII

wherein R_H, R_2, R_4 and W^* are as defined above. Those compound in which R_2 and/or R_4 designates methyl, ethyl, propyl, isopropyl, tert.-butyl, prop-2-enyl, 1,1-dimethylpropyl, 1,1-dimethylprop-2-enyl, 3-methylbutyl, or 3-methylbut-2-enyl are especially interesting.

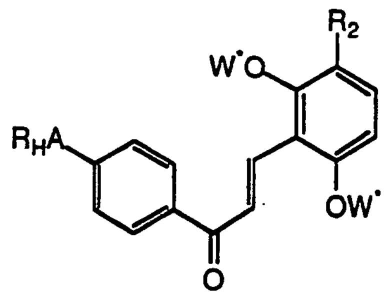
Other novel bis-aromatic α,β-unsaturated ketones have the general formula XIX



XIX

wherein R_2 and W^* are as defined above. Among these, the compounds in which R_2 designates propyl, prop-2-enyl, 1,1-dimethylpropyl, or 1,1-dimethylprop-2-enyl are especially interesting.

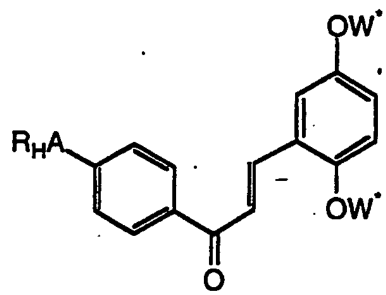
Another interesting class of novel bis-aromatic α,β-unsaturated ketones have the general formula XX



XX

wherein A, R_H, and R_2 is as defined above. Among these, the ones in which R_2 designates propyl, prop-2-enyl, 1,1-dimethylpropyl, 1,1-dimethylprop-2-enyl, 3-methylbutyl, or 3-methylbut-2-enyl are especially interesting.

Also, the bis-aromatic α,β-unsaturated ketones of the general formula XXI



XXI

wherein A, R_H, and W* are as defined above, are interesting novel compounds.

Specific examples of these novel bis-aromatic α,β-unsaturated ketones are the ones selected from

2,4-dimethoxy-4'-hydroxy-chalcone,
2,4-diethoxy-4'-hydroxy-chalcone,
2,4-di-n-propoxy-4'-hydroxy-chalcone,
2,4-diisopropoxy-4'-hydroxy-chalcone,
2,4-di-n-butoxy-4'-hydroxy-chalcone,
2,4-di-t-butoxy-4'-hydroxy-chalcone,
2,4-dimethoxy-4'-thiolo-chalcone,
2,4-diethoxy-4'-thiolo-chalcone,
2,4-di-n-propoxy-4'-thiolo-chalcone,
2,4-diisopropoxy-4'-thiolo-chalcone,

2,4-di-n-butoxy-4'-thiolo-chalcone,
2,4-di-t-butoxy-4'-thiolo-chalcone,
2,4-dimethoxy-4'-amino-chalcone,
2,4-diethoxy-4'-amino-chalcone,
2,4-di-n-propoxy-4'-amino-chalcone,
2,4-diisopropoxy-4'-amino-chalcone,
2,4-di-n-butoxy-4'-amino-chalcone,
2,4-di-t-butoxy-4'-amino-chalcone,
2,4-dimethoxy-4'-methylamino-chalcone,
2,4-diethoxy-4'-methylamino-chalcone,
2,4-di-n-propoxy-4'-methylamino-chalcone,
2,4-diisopropoxy-4'-methylamino-chalcone,
2,4-di-n-butoxy-4'-methylamino-chalcone,
2,4-di-t-butoxy-4'-methylamino-chalcone,
2,4-dimethoxy-5-methyl-4'-hydroxy-chalcone,
2,4-diethoxy-5-methyl-4'-hydroxy-chalcone,
2,4-di-n-propoxy-5-methyl-4'-hydroxy-chalcone,
2,4-diisopropoxy-5-methyl-4'-hydroxy-chalcone,
2,4-di-n-butoxy-5-methyl-4'-hydroxy-chalcone,
2,4-di-t-butoxy-5-methyl-4'-hydroxy-chalcone,
2,4-dimethoxy-5-methyl-4'-thiolo-chalcone,
2,4-diethoxy-5-methyl-4'-thiolo-chalcone,
2,4-di-n-propoxy-5-methyl-4'-thiolo-chalcone,
2,4-diisopropoxy-5-methyl-4'-thiolo-chalcone,
2,4-di-n-butoxy-5-methyl-4'-thiolo-chalcone,
2,4-di-t-butoxy-5-methyl-4'-thiolo-chalcone,
2,4-dimethoxy-5-methyl-4'-amino-chalcone,
2,4-diethoxy-5-methyl-4'-amino-chalcone,
2,4-di-n-propoxy-5-methyl-4'-amino-chalcone,
2,4-diisopropoxy-5-methyl-4'-amino-chalcone,
2,4-di-n-butoxy-5-methyl-4'-amino-chalcone,
2,4-di-t-butoxy-5-methyl-4'-amino-chalcone,
2,4-dimethoxy-5-methyl-4'-methylamino-chalcone,
2,4-diethoxy-5-methyl-4'-methylamino-chalcone,
2,4-di-n-propoxy-5-methyl-4'-methylamino-chalcone,
2,4-diisopropoxy-5-methyl-4'-methylamino-chalcone,
2,4-di-n-butoxy-5-methyl-4'-methylamino-chalcone,
2,4-di-t-butoxy-5-methyl-4'-methylamino-chalcone,
2,4-dimethoxy-5-prop-2-enyl-4'-hydroxy-chalcone,
2,4-diethoxy-5-prop-2-enyl-4'-hydroxy-chalcone,
2,4-di-n-propoxy-5-prop-2-enyl-4'-hydroxy-chalcone,

2,4-diisopropoxy-5-prop-2-enyl-4'-hydroxy-chalcone,
2,4-di-n-butoxy-5-prop-2-enyl-4'-hydroxy-chalcone,
2,4-di-t-butoxy-5-prop-2-enyl-4'-hydroxy-chalcone,
2,4-dimethoxy-5-prop-2-enyl-4'-thiolo-chalcone,
2,4-diethoxy-5-prop-2-enyl-4'-thiolo-chalcone,
2,4-di-n-propoxy-5-prop-2-enyl-4'-thiolo-chalcone,
2,4-diisopropoxy-5-prop-2-enyl-4'-thiolo-chalcone,
2,4-di-n-butoxy-5-prop-2-enyl-4'-thiolo-chalcone,
2,4-di-t-butoxy-5-prop-2-enyl-4'-thiolo-chalcone,
2,4-dimethoxy-5-prop-2-enyl-4'-amino-chalcone,
2,4-diethoxy-5-prop-2-enyl-4'-amino-chalcone,
2,4-di-n-propoxy-5-prop-2-enyl-4'-amino-chalcone,
2,4-diisopropoxy-5-prop-2-enyl-4'-amino-chalcone,
2,4-di-n-butoxy-5-prop-2-enyl-4'-amino-chalcone,
2,4-di-t-butoxy-5-prop-2-enyl-4'-amino-chalcone,
2,4-dimethoxy-5-prop-2-enyl-4'-methylamino-chalcone,
2,4-diethoxy-5-prop-2-enyl-4'-methylamino-chalcone,
2,4-di-n-propoxy-5-prop-2-enyl-4'-methylamino-chalcone,
2,4-diisopropoxy-5-prop-2-enyl-4'-methylamino-chalcone,
2,4-di-n-butoxy-5-prop-2-enyl-4'-methylamino-chalcone,
2,4-di-t-butoxy-5-prop-2-enyl-4'-methylamino-chalcone,
2,4-dimethoxy-5-propyl-4'-hydroxy-chalcone,
2,4-diethoxy-5-propyl-4'-hydroxy-chalcone,
2,4-di-n-propoxy-5-propyl-4'-hydroxy-chalcone,
2,4-diisopropoxy-5-propyl-4'-hydroxy-chalcone,
2,4-di-n-butoxy-5-propyl-4'-hydroxy-chalcone,
2,4-di-t-butoxy-5-propyl-4'-hydroxy-chalcone,
2,4-dimethoxy-5-propyl-4'-thiolo-chalcone,
2,4-diethoxy-5-propyl-4'-thiolo-chalcone,
2,4-di-n-propoxy-5-propyl-4'-thiolo-chalcone,
2,4-diisopropoxy-5-propyl-4'-thiolo-chalcone,
2,4-di-n-butoxy-5-propyl-4'-thiolo-chalcone,
2,4-di-t-butoxy-5-propyl-4'-thiolo-chalcone,
2,4-dimethoxy-5-propyl-4'-amino-chalcone,
2,4-diethoxy-5-propyl-4'-amino-chalcone,
2,4-di-n-propoxy-5-propyl-4'-amino-chalcone,
2,4-diisopropoxy-5-propyl-4'-amino-chalcone,
2,4-di-n-butoxy-5-propyl-4'-amino-chalcone,
2,4-di-t-butoxy-5-propyl-4'-amino-chalcone,
2,4-dimethoxy-5-propyl-4'-methylamino-chalcone,
2,4-diethoxy-5-propyl-4'-methylamino-chalcone,

2,4-di-n-propoxy-5-propyl-4'-methylamino-chalcone,
2,4-diisopropoxy-5-propyl-4'-methylamino-chalcone,
2,4-di-n-butoxy-5-propyl-4'-methylamino-chalcone,
2,4-di-t-butoxy-5-propyl-4'-methylamino-chalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-hydroxy-chalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-hydroxy-chalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-hydroxy-chalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-hydroxy-chalcone,
2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-hydroxy-chalcone,
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-hydroxy-chalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-thiolo-chalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-thiolo-chalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-thiolo-chalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-thiolo-chalcone,
2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-thiolo-chalcone,
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-thiolo-chalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-amino-chalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-amino-chalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-amino-chalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-amino-chalcone,
2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-amino-chalcone,
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-amino-chalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-methylamino-chalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-methylamino-chalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-methylamino-chalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-methylamino-chalcone,
2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-methylamino-chalcone,
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-methylamino-chalcone,
2,4-dimethoxy-5-(1,1-dimethylprop-2-enyl)-4'-hydroxy-chalcone,
2,4-diethoxy-5-(1,1-dimethylprop-2-enyl)-4'-hydroxy-chalcone,
2,4-di-n-propoxy-5-(1,1-dimethylprop-2-enyl)-4'-hydroxy-chalcone,
2,4-diisopropoxy-5-(1,1-dimethylprop-2-enyl)-4'-hydroxy-chalcone,
2,4-di-n-butoxy-5-(1,1-dimethylprop-2-enyl)-4'-hydroxy-chalcone,
2,4-di-t-butoxy-5-(1,1-dimethylprop-2-enyl)-4'-hydroxy-chalcone,
2,4-dimethoxy-5-(1,1-dimethylprop-2-enyl)-4'-thiolo-chalcone,
2,4-diethoxy-5-(1,1-dimethylprop-2-enyl)-4'-thiolo-chalcone,
2,4-di-n-propoxy-5-(1,1-dimethylprop-2-enyl)-4'-thiolo-chalcone,
2,4-diisopropoxy-5-(1,1-dimethylprop-2-enyl)-4'-thiolo-chalcone,
2,4-di-n-butoxy-5-(1,1-dimethylprop-2-enyl)-4'-thiolo-chalcone,
2,4-di-t-butoxy-5-(1,1-dimethylprop-2-enyl)-4'-thiolo-chalcone,
2,4-dimethoxy-5-(1,1-dimethylprop-2-enyl)-4'-amino-chalcone,

2,4-diethoxy-5-(1,1-dimethylprop-2-enyl)-4'-amino-chalcone,
2,4-di-n-propoxy-5-(1,1-dimethylprop-2-enyl)-4'-amino-chalcone,
2,4-diisopropoxy-5-(1,1-dimethylprop-2-enyl)-4'-amino-chalcone,
2,4-di-n-butoxy-5-(1,1-dimethylprop-2-enyl)-4'-amino-chalcone,
2,4-di-t-butoxy-5-(1,1-dimethylprop-2-enyl)-4'-amino-chalcone,
2,4-dimethoxy-5-(1,1-dimethylprop-2-enyl)-4'-methylamino-chalcone,
2,4-diethoxy-5-(1,1-dimethylprop-2-enyl)-4'-methylamino-chalcone,
2,4-di-n-propoxy-5-(1,1-dimethylprop-2-enyl)-4'-methylamino-chalcone,

2,4-diisopropoxy-5-(1,1-dimethylprop-2-enyl)-4'-methylamino-chalcone,
2,4-di-n-butoxy-5-(1,1-dimethylprop-2-enyl)-4'-methylamino-chalcone,
2,4-di-t-butoxy-5-(1,1-dimethylprop-2-enyl)-4'-methylamino-chalcone,

and the corresponding ketones in which Z is one of the groups (A)-(E) defined above in particular pivaolyloxymethyl or N,N-dimethylcarbamoyl, such as

2,4-dimethoxy-4'-pivaloyloxy-chalcone,
2,4-diethoxy-4'-pivaloyloxy-chalcone,
2,4-di-n-propoxy-4'-pivaloyloxy-chalcone,
2,4-diisopropoxy-4'-pivaloyloxy-chalcone,
2,4-di-n-butoxy-4'-pivaloyloxy-chalcone,
2,4-di-t-butoxy-4'-pivaloyloxy-chalcone,
2,4-dimethoxy-5-methyl-4'-pivaloyloxy-chalcone,
2,4-diethoxy-5-methyl-4'-pivaloyloxy-chalcone,
2,4-di-n-propoxy-5-methyl-4'-pivaloyloxy-chalcone,
2,4-diisopropoxy-5-methyl-4'-pivaloyloxy-chalcone,
2,4-di-n-butoxy-5-methyl-4'-pivaloyloxy-chalcone,
2,4-di-t-butoxy-5-methyl-4'-pivaloyloxy-chalcone,
2,4-dimethoxy-5-prop-2-enyl-4'-pivaloyloxy-chalcone,
2,4-diethoxy-5-prop-2-enyl-4'-pivaloyloxy-chalcone,
2,4-di-n-propoxy-5-prop-2-enyl-4'-pivaloyloxy-chalcone,
2,4-diisopropoxy-5-prop-2-enyl-4'-pivaloyloxy-chalcone,
2,4-di-n-butoxy-5-prop-2-enyl-4'-pivaloyloxy-chalcone,
2,4-di-t-butoxy-5-prop-2-enyl-4'-pivaloyloxy-chalcone,
2,4-dimethoxy-5-propyl-4'-pivaloyloxy-chalcone,
2,4-diethoxy-5-propyl-4'-pivaloyloxy-chalcone,
2,4-di-n-propoxy-5-propyl-4'-pivaloyloxy-chalcone,
2,4-diisopropoxy-5-propyl-4'-pivaloyloxy-chalcone,
2,4-di-n-butoxy-5-propyl-4'-pivaloyloxy-chalcone,
2,4-di-t-butoxy-5-propyl-4'-pivaloyloxy-chalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxy-chalcone,

2,4-diethoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxy-chalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxy-chalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxy-chalcone,
2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxy-chalcone,
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxy-chalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxy-chalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxy-chalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxy-chalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxy-chalcone,
2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxy-chalcone,
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxy-chalcone,

2,4-dimethoxy-4'-pivaloyloxymethoxy-chalcone,
2,4-diethoxy-4'-pivaloyloxymethoxy-chalcone,
2,4-di-n-propoxy-4'-pivaloyloxymethoxy-chalcone,
2,4-diisopropoxy-4'-pivaloyloxymethoxy-chalcone,
2,4-di-n-butoxy-4'-pivaloyloxymethoxy-chalcone,
2,4-di-t-butoxy-4'-pivaloyloxymethoxy-chalcone,
2,4-dimethoxy-5-methyl-4'-pivaloyloxymethoxy-chalcone,
2,4-diethoxy-5-methyl-4'-pivaloyloxymethoxy-chalcone,
2,4-di-n-propoxy-5-methyl-4'-pivaloyloxymethoxy-chalcone,
2,4-diisopropoxy-5-methyl-4'-pivaloyloxymethoxy-chalcone,
2,4-di-n-butoxy-5-methyl-4'-pivaloyloxymethoxy-chalcone,
2,4-di-t-butoxy-5-methyl-4'-pivaloyloxymethoxy-chalcone,
2,4-dimethoxy-5-prop-2-enyl-4'-pivaloyloxymethoxy-chalcone,
2,4-diethoxy-5-prop-2-enyl-4'-pivaloyloxymethoxy-chalcone,
2,4-di-n-propoxy-5-prop-2-enyl-4'-pivaloyloxymethoxy-chalcone,
2,4-diisopropoxy-5-prop-2-enyl-4'-pivaloyloxymethoxy-chalcone,
2,4-di-n-butoxy-5-prop-2-enyl-4'-pivaloyloxymethoxy-chalcone,
2,4-di-t-butoxy-5-prop-2-enyl-4'-pivaloyloxymethoxy-chalcone,
2,4-dimethoxy-5-propyl-4'-pivaloyloxymethoxy-chalcone,
2,4-diethoxy-5-propyl-4'-pivaloyloxymethoxy-chalcone,
2,4-di-n-propoxy-5-propyl-4'-pivaloyloxymethoxy-chalcone,
2,4-diisopropoxy-5-propyl-4'-pivaloyloxymethoxy-chalcone,
2,4-di-n-butoxy-5-propyl-4'-pivaloyloxymethoxy-chalcone,
2,4-di-t-butoxy-5-propyl-4'-pivaloyloxymethoxy-chalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxymethoxy-chalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxymethoxy-chalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxymethoxy-chalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxymethoxy-chalcone,
2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxymethoxy-chalcone,

2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxymethoxy-chalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxymethoxy-chalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxymethoxy-chalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxymethoxy-chalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxymethoxy-chalcone,
2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxymethoxy-chalcone,
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxymethoxy-chalcone,

2,4-dimethoxy-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-diethoxy-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-di-n-propoxy-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-diisopropoxy-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-di-n-butoxy-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-di-t-butoxy-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-dimethoxy-5-methyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-diethoxy-5-methyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-di-n-propoxy-5-methyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-diisopropoxy-5-methyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-di-n-butoxy-5-methyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-di-t-butoxy-5-methyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-dimethoxy-5-prop-2-enyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-diethoxy-5-prop-2-enyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-di-n-propoxy-5-prop-2-enyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-diisopropoxy-5-prop-2-enyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-di-n-butoxy-5-prop-2-enyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-di-t-butoxy-5-prop-2-enyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-dimethoxy-5-propyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-diethoxy-5-propyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-di-n-propoxy-5-propyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-diisopropoxy-5-propyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-di-n-butoxy-5-propyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-di-t-butoxy-5-propyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-(N,N-dimethylcarbamoyl)-chalcone,

2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-(N,N-dimethylcarbamoyl)-chalcone.

Specific examples of bis-aromatic α,β-unsaturated ketones are:
2-methoxy-4,4'-di-pivaloyloxy-5-methyl-chalcone,
2-methoxy-4,4'-di-pivaloyloxy-5-ethyl-chalcone,
2-methoxy-4,4'-di-pivaloyloxy-5-propyl-chalcone,
2-methoxy-4,4'-di-pivaloyloxy-5-prop-2-enyl-chalcone,
2-methoxy-4,4'-di-pivaloyloxy-5-(1,1-dimethylprop-2-enyl)-chalcone,
2-methoxy-4,4'-di-pivaloyloxy-5-(1,1-dimethylethyl)-chalcone,
2-methoxy-4,4'-di-pivaloyloxymethoxy-5-methyl-chalcone,
2-methoxy-4,4'-di-pivaloyloxymethoxy-5-ethyl-chalcone,
2-methoxy-4,4'-di-pivaloyloxymethoxy-5-propyl-chalcone,
2-methoxy-4,4'-di-pivaloyloxymethoxy-5-propenyl-chalcone,
2-methoxy-4,4'-di-pivaloyloxymethoxy-5-(1,1-dimethylprop-2-enyl)-chalcone,
2-methoxy-4,4'-di-pivaloyloxymethoxy-5-(1,1-dimethylethyl)-chalcone,
2-methoxy-4,4'-di-(N,N-dimethylcarbamoyl)-5-methyl-chalcone,
2-methoxy-4,4'-di-(N,N-dimethylcarbamoyl)-5-ethyl-chalcone,
2-methoxy-4,4'-di-(N,N-dimethylcarbamoyl)-5-propyl-chalcone,
2-methoxy-4,4'-di-(N,N-dimethylcarbamoyl)-5-propenyl-chalcone,
2-methoxy-4,4'-di-(N,N-dimethylcarbamoyl)-5-(1,1-dimethylprop-2-enyl)-chalcone,
2-methoxy-4,4'-di-(N,N-dimethylcarbamoyl)-5-(1,1-dimethylethyl)-chalcone,
2-methoxy-4,4'-di-methoxymethoxy-5-methyl-chalcone,
2-methoxy-4,4'-di-methoxymethoxy-5-ethyl-chalcone,
2-methoxy-4,4'-di-methoxymethoxy-5-propyl-chalcone,
2-methoxy-4,4'-di-methoxymethoxy-5-prop-2-enyl-chalcone,
2-methoxy-4,4'-di-methoxymethoxy-5-(1,1-dimethylpropenyl)-chalcone,
2-methoxy-4,4'-di-methoxymethoxy-5-(1,1-dimethylethyl)-chalcone,
2-methoxy-4,4'-di-propenoxy-5-methyl-chalcone,
2-methoxy-4,4'-di-propenoxy-5-ethyl-chalcone,
2-methoxy-4,4'-di-propenoxy-5-propyl-chalcone,
2-methoxy-4,4'-di-propenoxy-5-prop-2-enyl-chalcone,
2-methoxy-4,4'-di-propenoxy-5-(1,1-dimethylpropenyl)-chalcone, and
2-methoxy-4,4'-di-propenoxy-5-(1,1-dimethylethyl)-chalcone.

2,4-dimethoxy-4'-(2-prop-2-enyloxy)-chalcone,
2,4-diethoxy-4'-(2-prop-2-enyloxy)-chalcone,
2,4-di-n-propoxy-4'-(2-prop-2-enyloxy)-chalcone,
2,4-diisopropoxy-4'-(2-prop-2-enyloxy)-chalcone,
2,4-di-n-butoxy-4'-(2-prop-2-enyloxy)-chalcone,
2,4-di-t-butoxy-4'-(2-prop-2-enyloxy)-chalcone,

2,4-dimethoxy-5-methyl-4'-(2-prop-2-enyloxy)-chalcone,
2,4-diethoxy-5-methyl-4'-(2-prop-2-enyloxy)-chalcone,
2,4-di-n-propoxy-5-methyl-4'-(2-prop-2-enyloxy)-chalcone,
2,4-diisopropoxy-5-methyl-4'-(2-prop-2-enyloxy)-chalcone,
2,4-di-n-butoxy-5-methyl-4'-(2-prop-2-enyloxy)-chalcone,
2,4-di-t-butoxy-5-methyl-4'-(2-prop-2-enyloxy)-chalcone,
2,4-dimethoxy-5-prop-2-enyl-4'-(2-prop-2-enyloxy)-chalcone,
2,4-diethoxy-5-prop-2-enyl-4'-(2-prop-2-enyloxy)-chalcone,
2,4-di-n-propoxy-5-prop-2-enyl-4'-(2-prop-2-enyloxy)-chalcone,
2,4-diisopropoxy-5-prop-2-enyl-4'-(2-prop-2-enyloxy)-chalcone,
2,4-di-n-butoxy-5-prop-2-enyl-4'-(2-prop-2-enyloxy)-chalcone,
2,4-di-t-butoxy-5-prop-2-enyl-4'-(2-prop-2-enyloxy)-chalcone,
2,4-dimethoxy-5-propyl-4'-(2-prop-2-enyloxy)-chalcone,
2,4-diethoxy-5-propyl-4'-(2-prop-2-enyloxy)-chalcone,
2,4-di-n-propoxy-5-propyl-4'-(2-prop-2-enyloxy)-chalcone,
2,4-diisopropoxy-5-propyl-4'-(2-prop-2-enyloxy)-chalcone,
2,4-di-n-butoxy-5-propyl-4'-(2-prop-2-enyloxy)-chalcone,
2,4-di-t-butoxy-5-propyl-4'-(2-prop-2-enyloxy)-chalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-(2-prop-2-enyloxy)-chalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-(2-prop-2-enyloxy)-chalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-(2-prop-2-enyloxy)-chalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-(2-prop-2-enyloxy)-chalcone,
2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-(2-prop-2-enyloxy)-chalcone,
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-(2-prop-2-enyloxy)-chalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-(2-prop-2-enyloxy)-chalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-(2-prop-2-enyloxy)-chalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-(2-prop-2-enyloxy)-chalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-(2-prop-2-enyloxy)-chalcone,
2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-(2-prop-2-enyloxy)-chalcone,
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-(2-prop-2-enyloxy)-chalcone.

2,6-dimethoxy-4'-hydroxy-chalcone,
2,6-diethoxy-4'-hydroxy-chalcone,
2,6-di-n-propoxy-4'-hydroxy-chalcone,
2,6-diisopropoxy-4'-hydroxy-chalcone,
2,6-di-n-butoxy-4'-hydroxy-chalcone,
2,6-di-t-butoxy-4'-hydroxy-chalcone,
2,6-dimethoxy-4'-thiolo-chalcone,
2,6-diethoxy-4'-thiolo-chalcone,
2,6-di-n-propoxy-4'-thiolo-chalcone,
2,6-diisopropoxy-4'-thiolo-chalcone,

2,6-di-n-butoxy-4'-thiolo-chalcone,
2,6-di-t-butoxy-4'-thiolo-chalcone,
2,6-dimethoxy-4'-amino-chalcone,
2,6-diethoxy-4'-amino-chalcone,
2,6-di-n-propoxy-4'-amino-chalcone,
2,6-diisopropoxy-4'-amino-chalcone,
2,6-di-n-butoxy-4'-amino-chalcone,
2,6-di-t-butoxy-4'-amino-chalcone,
2,6-dimethoxy-4'-methylamino-chalcone,
2,6-diethoxy-4'-methylamino-chalcone,
2,6-di-n-propoxy-4'-methylamino-chalcone,
2,6-diisopropoxy-4'-methylamino-chalcone,
2,6-di-n-butoxy-4'-methylamino-chalcone,
2,6-di-t-butoxy-4'-methylamino-chalcone,
2,6-dimethoxy-5-methyl-4'-hydroxy-chalcone,
2,6-diethoxy-5-methyl-4'-hydroxy-chalcone,
2,6-di-n-propoxy-5-methyl-4'-hydroxy-chalcone,
2,6-diisopropoxy-5-methyl-4'-hydroxy-chalcone,
2,6-di-n-butoxy-5-methyl-4'-hydroxy-chalcone,
2,6-di-t-butoxy-5-methyl-4'-hydroxy-chalcone,
2,6-dimethoxy-5-methyl-4'-thiolo-chalcone,
2,6-diethoxy-5-methyl-4'-thiolo-chalcone,
2,6-di-n-propoxy-5-methyl-4'-thiolo-chalcone,
2,6-diisopropoxy-5-methyl-4'-thiolo-chalcone,
2,6-di-n-butoxy-5-methyl-4'-thiolo-chalcone,
2,6-di-t-butoxy-5-methyl-4'-thiolo-chalcone,
2,6-dimethoxy-5-methyl-4'-amino-chalcone,
2,6-diethoxy-5-methyl-4'-amino-chalcone,
2,6-di-n-propoxy-5-methyl-4'-amino-chalcone,
2,6-diisopropoxy-5-methyl-4'-amino-chalcone,
2,6-di-n-butoxy-5-methyl-4'-amino-chalcone,
2,6-di-t-butoxy-5-methyl-4'-amino-chalcone,
2,6-dimethoxy-5-methyl-4'-methylamino-chalcone,
2,6-diethoxy-5-methyl-4'-methylamino-chalcone,
2,6-di-n-propoxy-5-methyl-4'-methylamino-chalcone,
2,6-diisopropoxy-5-methyl-4'-methylamino-chalcone,
2,6-di-n-butoxy-5-methyl-4'-methylamino-chalcone,
2,6-di-t-butoxy-5-methyl-4'-methylamino-chalcone,
2,6-dimethoxy-5-prop-2-enyl-4'-hydroxy-chalcone,
2,6-diethoxy-5-prop-2-enyl-4'-hydroxy-chalcone,
2,6-di-n-propoxy-5-prop-2-enyl-4'-hydroxy-chalcone,

2,6-diisopropoxy-5-prop-2-enyl-4'-hydroxy-chalcone,
2,6-di-n-butoxy-5-prop-2-enyl-4'-hydroxy-chalcone,
2,6-di-t-butoxy-5-prop-2-enyl-4'-hydroxy-chalcone,
2,6-dimethoxy-5-prop-2-enyl-4'-thiolo-chalcone,
2,6-diethoxy-5-prop-2-enyl-4'-thiolo-chalcone,
2,6-di-n-propoxy-5-prop-2-enyl-4'-thiolo-chalcone,
2,6-diisopropoxy-5-prop-2-enyl-4'-thiolo-chalcone,
2,6-di-n-butoxy-5-prop-2-enyl-4'-thiolo-chalcone,
2,6-di-t-butoxy-5-prop-2-enyl-4'-thiolo-chalcone,
2,6-dimethoxy-5-prop-2-enyl-4'-amino-chalcone,
2,6-diethoxy-5-prop-2-enyl-4'-amino-chalcone,
2,6-di-n-propoxy-5-prop-2-enyl-4'-amino-chalcone,
2,6-diisopropoxy-5-prop-2-enyl-4'-amino-chalcone,
2,6-di-n-butoxy-5-prop-2-enyl-4'-amino-chalcone,
2,6-di-t-butoxy-5-prop-2-enyl-4'-amino-chalcone,
2,6-dimethoxy-5-prop-2-enyl-4'-methylamino-chalcone,
2,6-diethoxy-5-prop-2-enyl-4'-methylamino-chalcone,
2,6-di-n-propoxy-5-prop-2-enyl-4'-methylamino-chalcone,
2,6-diisopropoxy-5-prop-2-enyl-4'-methylamino-chalcone,
2,6-di-n-butoxy-5-prop-2-enyl-4'-methylamino-chalcone,
2,6-di-t-butoxy-5-prop-2-enyl-4'-methylamino-chalcone,
2,6-dimethoxy-5-propyl-4'-hydroxy-chalcone,
2,6-diethoxy-5-propyl-4'-hydroxy-chalcone,
2,6-di-n-propoxy-5-propyl-4'-hydroxy-chalcone,
2,6-diisopropoxy-5-propyl-4'-hydroxy-chalcone,
2,6-di-n-butoxy-5-propyl-4'-hydroxy-chalcone,
2,6-di-t-butoxy-5-propyl-4'-hydroxy-chalcone,
2,6-dimethoxy-5-propyl-4'-thiolo-chalcone,
2,6-diethoxy-5-propyl-4'-thiolo-chalcone,
2,6-di-n-propoxy-5-propyl-4'-thiolo-chalcone,
2,6-diisopropoxy-5-propyl-4'-thiolo-chalcone,
2,6-di-n-butoxy-5-propyl-4'-thiolo-chalcone,
2,6-di-t-butoxy-5-propyl-4'-thiolo-chalcone,
2,6-dimethoxy-5-propyl-4'-amino-chalcone,
2,6-diethoxy-5-propyl-4'-amino-chalcone,
2,6-di-n-propoxy-5-propyl-4'-amino-chalcone,
2,6-diisopropoxy-5-propyl-4'-amino-chalcone,
2,6-di-n-butoxy-5-propyl-4'-amino-chalcone,
2,6-di-t-butoxy-5-propyl-4'-amino-chalcone,
2,6-dimethoxy-5-propyl-4'-methylamino-chalcone,
2,6-diethoxy-5-propyl-4'-methylamino-chalcone,

2,6-di-n-propoxy-5-propyl-4'-methylamino-chalcone,
2,6-diisopropoxy-5-propyl-4'-methylamino-chalcone,
2,6-di-n-butoxy-5-propyl-4'-methylamino-chalcone,
2,6-di-t-butoxy-5-propyl-4'-methylamino-chalcone,
2,6-dimethoxy-5-(1,1-dimethylethyl)-4'-hydroxy-chalcone,
2,6-diethoxy-5-(1,1-dimethylethyl)-4'-hydroxy-chalcone,
2,6-di-n-propoxy-5-(1,1-dimethylethyl)-4'-hydroxy-chalcone,
2,6-diisopropoxy-5-(1,1-dimethylethyl)-4'-hydroxy-chalcone,
2,6-di-n-butoxy-5-(1,1-dimethylethyl)-4'-hydroxy-chalcone,
2,6-di-t-butoxy-5-(1,1-dimethylethyl)-4'-hydroxy-chalcone,
2,6-dimethoxy-5-(1,1-dimethylethyl)-4'-thiolo-chalcone,
2,6-diethoxy-5-(1,1-dimethylethyl)-4'-thiolo-chalcone,
2,6-di-n-propoxy-5-(1,1-dimethylethyl)-4'-thiolo-chalcone,
2,6-diisopropoxy-5-(1,1-dimethylethyl)-4'-thiolo-chalcone,
2,6-di-n-butoxy-5-(1,1-dimethylethyl)-4'-thiolo-chalcone,
2,6-di-t-butoxy-5-(1,1-dimethylethyl)-4'-thiolo-chalcone,
2,6-dimethoxy-5-(1,1-dimethylethyl)-4'-amino-chalcone,
2,6-diethoxy-5-(1,1-dimethylethyl)-4'-amino-chalcone,
2,6-di-n-propoxy-5-(1,1-dimethylethyl)-4'-amino-chalcone,
2,6-diisopropoxy-5-(1,1-dimethylethyl)-4'-amino-chalcone,
2,6-di-n-butoxy-5-(1,1-dimethylethyl)-4'-amino-chalcone,
2,6-di-t-butoxy-5-(1,1-dimethylethyl)-4'-amino-chalcone,
2,6-dimethoxy-5-(1,1-dimethylethyl)-4'-methylamino-chalcone,
2,6-diethoxy-5-(1,1-dimethylethyl)-4'-methylamino-chalcone,
2,6-di-n-propoxy-5-(1,1-dimethylethyl)-4'-methylamino-chalcone,
2,6-diisopropoxy-5-(1,1-dimethylethyl)-4'-methylamino-chalcone,
2,6-di-n-butoxy-5-(1,1-dimethylethyl)-4'-methylamino-chalcone,
2,6-di-t-butoxy-5-(1,1-dimethylethyl)-4'-methylamino-chalcone,
2,6-dimethoxy-5-(1,1-dimethylprop-2-enyl)-4'-hydroxy-chalcone,
2,6-diethoxy-5-(1,1-dimethylprop-2-enyl)-4'-hydroxy-chalcone,
2,6-di-n-propoxy-5-(1,1-dimethylprop-2-enyl)-4'-hydroxy-chalcone,
2,6-diisopropoxy-5-(1,1-dimethylprop-2-enyl)-4'-hydroxy-chalcone,
2,6-di-n-butoxy-5-(1,1-dimethylprop-2-enyl)-4'-hydroxy-chalcone,
2,6-di-t-butoxy-5-(1,1-dimethylprop-2-enyl)-4'-hydroxy-chalcone,
2,6-dimethoxy-5-(1,1-dimethylprop-2-enyl)-4'-thiolo-chalcone,
2,6-diethoxy-5-(1,1-dimethylprop-2-enyl)-4'-thiolo-chalcone,
2,6-di-n-propoxy-5-(1,1-dimethylprop-2-enyl)-4'-thiolo-chalcone,
2,6-diisopropoxy-5-(1,1-dimethylprop-2-enyl)-4'-thiolo-chalcone,
2,6-di-n-butoxy-5-(1,1-dimethylprop-2-enyl)-4'-thiolo-chalcone,
2,6-di-t-butoxy-5-(1,1-dimethylprop-2-enyl)-4'-thiolo-chalcone,
2,6-dimethoxy-5-(1,1-dimethylprop-2-enyl)-4'-amino-chalcone,

2,6-diethoxy-5-(1,1-dimethylprop-2-enyl)-4'-amino-chalcone,
2,6-di-n-propoxy-5-(1,1-dimethylprop-2-enyl)-4'-amino-chalcone,
2,6-diisopropoxy-5-(1,1-dimethylprop-2-enyl)-4'-amino-chalcone,
2,6-di-n-butoxy-5-(1,1-dimethylprop-2-enyl)-4'-amino-chalcone,
2,6-di-t-butoxy-5-(1,1-dimethylprop-2-enyl)-4'-amino-chalcone,
2,6-dimethoxy-5-(1,1-dimethylprop-2-enyl)-4'-methylamino-chalcone,
2,6-diethoxy-5-(1,1-dimethylprop-2-enyl)-4'-methylamino-chalcone,
2,6-di-n-propoxy-5-(1,1-dimethylprop-2-enyl)-4'-methylamino-chalcone,

2,6-diisopropoxy-5-(1,1-dimethylprop-2-enyl)-4'-methylamino-chalcone,
2,6-di-n-butoxy-5-(1,1-dimethylprop-2-enyl)-4'-methylamino-chalcone,
2,6-di-t-butoxy-5-(1,1-dimethylprop-2-enyl)-4'-methylamino-chalcone,

and the corresponding ketones in which Z is one of the groups (A)-(E) defined above, in particular pivaolyloxymethyl or N,N-dimethylcarbamoyl, such as

2,6-dimethoxy-4'-pivaloyloxy-chalcone,
2,6-diethoxy-4'-pivaloyloxy-chalcone,
2,6-di-n-propoxy-4'-pivaloyloxy-chalcone,
2,6-diisopropoxy-4'-pivaloyloxy-chalcone,
2,6-di-n-butoxy-4'-pivaloyloxy-chalcone,
2,6-di-t-butoxy-4'-pivaloyloxy-chalcone,
2,6-dimethoxy-5-methyl-4'-pivaloyloxy-chalcone,
2,6-diethoxy-5-methyl-4'-pivaloyloxy-chalcone,
2,6-di-n-propoxy-5-methyl-4'-pivaloyloxy-chalcone,
2,6-diisopropoxy-5-methyl-4'-pivaloyloxy-chalcone,
2,6-di-n-butoxy-5-methyl-4'-pivaloyloxy-chalcone,
2,6-di-t-butoxy-5-methyl-4'-pivaloyloxy-chalcone,
2,6-dimethoxy-5-prop-2-enyl-4'-pivaloyloxy-chalcone,
2,6-diethoxy-5-prop-2-enyl-4'-pivaloyloxy-chalcone,
2,6-di-n-propoxy-5-prop-2-enyl-4'-pivaloyloxy-chalcone,
2,6-diisopropoxy-5-prop-2-enyl-4'-pivaloyloxy-chalcone,
2,6-di-n-butoxy-5-prop-2-enyl-4'-pivaloyloxy-chalcone,
2,6-di-t-butoxy-5-prop-2-enyl-4'-pivaloyloxy-chalcone,
2,6-dimethoxy-5-propyl-4'-pivaloyloxy-chalcone,
2,6-diethoxy-5-propyl-4'-pivaloyloxy-chalcone,
2,6-di-n-propoxy-5-propyl-4'-pivaloyloxy-chalcone,
2,6-diisopropoxy-5-propyl-4'-pivaloyloxy-chalcone,
2,6-di-n-butoxy-5-propyl-4'-pivaloyloxy-chalcone,
2,6-di-t-butoxy-5-propyl-4'-pivaloyloxy-chalcone,
2,6-dimethoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxy-chalcone,.

2,6-diethoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxy-chalcone,
2,6-di-n-propoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxy-chalcone,
2,6-diisopropoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxy-chalcone,
2,6-di-n-butoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxy-chalcone,
2,6-di-t-butoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxy-chalcone,
2,6-dimethoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxy-chalcone,
2,6-diethoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxy-chalcone,
2,6-di-n-propoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxy-chalcone,
2,6-diisopropoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxy-chalcone,
2,6-di-n-butoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxy-chalcone,
2,6-di-t-butoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxy-chalcone,

2,6-dimethoxy-4'-pivaloyloxymethoxy-chalcone,
2,6-diethoxy-4'-pivaloyloxymethoxy-chalcone,
2,6-di-n-propoxy-4'-pivaloyloxymethoxy-chalcone,
2,6-diisopropoxy-4'-pivaloyloxymethoxy-chalcone,
2,6-di-n-butoxy-4'-pivaloyloxymethoxy-chalcone,
2,6-di-t-butoxy-4'-pivaloyloxymethoxy-chalcone,
2,6-dimethoxy-5-methyl-4'-pivaloyloxymethoxy-chalcone,
2,6-diethoxy-5-methyl-4'-pivaloyloxymethoxy-chalcone,
2,6-di-n-propoxy-5-methyl-4'-pivaloyloxymethoxy-chalcone,
2,6-diisopropoxy-5-methyl-4'-pivaloyloxymethoxy-chalcone,
2,6-di-n-butoxy-5-methyl-4'-pivaloyloxymethoxy-chalcone,
2,6-di-t-butoxy-5-methyl-4'-pivaloyloxymethoxy-chalcone,
2,6-dimethoxy-5-prop-2-enyl-4'-pivaloyloxymethoxy-chalcone,
2,6-diethoxy-5-prop-2-enyl-4'-pivaloyloxymethoxy-chalcone,
2,6-di-n-propoxy-5-prop-2-enyl-4'-pivaloyloxymethoxy-chalcone,
2,6-diisopropoxy-5-prop-2-enyl-4'-pivaloyloxymethoxy-chalcone,
2,6-di-n-butoxy-5-prop-2-enyl-4'-pivaloyloxymethoxy-chalcone,
2,6-di-t-butoxy-5-prop-2-enyl-4'-pivaloyloxymethoxy-chalcone,
2,6-dimethoxy-5-propyl-4'-pivaloyloxymethoxy-chalcone,
2,6-diethoxy-5-propyl-4'-pivaloyloxymethoxy-chalcone,
2,6-di-n-propoxy-5-propyl-4'-pivaloyloxymethoxy-chalcone,
2,6-diisopropoxy-5-propyl-4'-pivaloyloxymethoxy-chalcone,
2,6-di-n-butoxy-5-propyl-4'-pivaloyloxymethoxy-chalcone,
2,6-di-t-butoxy-5-propyl-4'-pivaloyloxymethoxy-chalcone,
2,6-dimethoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxymethoxy-chalcone,
2,6-diethoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxymethoxy-chalcone,
2,6-di-n-propoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxymethoxy-chalcone,
2,6-diisopropoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxymethoxy-chalcone,
2,6-di-n-butoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxymethoxy-chalcone,

2,6-di-t-butoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxymethoxy-chalcone,
2,6-dimethoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxymethoxy-chalcone,
2,6-diethoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxymethoxy-chalcone,
2,6-di-n-propoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxymethoxy-chalcone,
2,6-diisopropoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxymethoxy-chalcone,
2,6-di-n-butoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxymethoxy-chalcone,
2,6-di-t-butoxy-5-(1,1-dimethylethyl)-4'-pivaloyloxymethoxy-chalcone,

2,6-dimethoxy-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-diethoxy-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-di-n-propoxy-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-diisopropoxy-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-di-n-butoxy-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-di-t-butoxy-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-dimethoxy-5-methyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-diethoxy-5-methyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-di-n-propoxy-5-methyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-diisopropoxy-5-methyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-di-n-butoxy-5-methyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-di-t-butoxy-5-methyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-dimethoxy-5-prop-2-enyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-diethoxy-5-prop-2-enyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-di-n-propoxy-5-prop-2-enyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-diisopropoxy-5-prop-2-enyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-di-n-butoxy-5-prop-2-enyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-di-t-butoxy-5-prop-2-enyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-dimethoxy-5-propyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-diethoxy-5-propyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-di-n-propoxy-5-propyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-diisopropoxy-5-propyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-di-n-butoxy-5-propyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-di-t-butoxy-5-propyl-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-dimethoxy-5-(1,1-dimethylethyl)-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-diethoxy-5-(1,1-dimethylethyl)-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-di-n-propoxy-5-(1,1-dimethylethyl)-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-diisopropoxy-5-(1,1-dimethylethyl)-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-di-n-butoxy-5-(1,1-dimethylethyl)-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-di-t-butoxy-5-(1,1-dimethylethyl)-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-dimethoxy-5-(1,1-dimethylethyl)-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-diethoxy-5-(1,1-dimethylethyl)-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-di-n-propoxy-5-(1,1-dimethylethyl)-4'-(N,N-dimethylcarbamoyl)-chalcone,

2,6-diisopropoxy-5-(1,1-dimethylethyl)-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-di-n-butoxy-5-(1,1-dimethylethyl)-4'-(N,N-dimethylcarbamoyl)-chalcone,
2,6-di-t-butoxy-5-(1,1-dimethylethyl)-4'-(N,N-dimethylcarbamoyl)-chalcone.

2,6-dimethoxy-4'-di-pivaloyloxy-3-methyl-chalcone,
2,6-dimethoxy-4'-di-pivaloyloxy-3-ethyl-chalcone,
2,6-dimethoxy-4'-di-pivaloyloxy-3-propyl-chalcone,
2,6-dimethoxy-4'-di-pivaloyloxy-3-prop-2-enyl-chalcone,
2,6-dimethoxy-4'-di-pivaloyloxy-3-(1,1-dimethylprop-2-enyl)-chalcone,
2,6-dimethoxy-4'-di-pivaloyloxy-3-(1,1-dimethylethyl)-chalcone,
2,6-dimethoxy-4'-di-pivaloyloxymethoxy-3-methyl-chalcone,
2,6-dimethoxy-4'-di-pivaloyloxymethoxy-3-ethyl-chalcone,
2,6-dimethoxy-4'-di-pivaloyloxymethoxy-3-propyl-chalcone,
2,6-dimethoxy-4'-di-pivaloyloxymethoxy-3-propenyl-chalcone,
2,6-dimethoxy-4'-di-pivaloyloxymethoxy-3-(1,1-dimethylprop-2-enyl)-chalcone,
2,6-dimethoxy-4'-di-pivaloyloxymethoxy-3-(1,1-dimethylethyl)-chalcone,
2,6-dimethoxy-4'-di-(N,N-dimethylcarbamoyl)-3-methyl-chalcone,
2,6-dimethoxy-4'-di-(N,N-dimethylcarbamoyl)-3-ethyl-chalcone,
2,6-dimethoxy-4'-di-(N,N-dimethylcarbamoyl)-3-propyl-chalcone,
2,6-dimethoxy-4'-di-(N,N-dimethylcarbamoyl)-3-propenyl-chalcone,
2,6-dimethoxy-4'-di-(N,N-dimethylcarbamoyl)-3-(1,1-dimethylprop-2-enyl)-chalcone,
2,6-dimethoxy-4'-di-(N,N-dimethylcarbamoyl)-3-(1,1-dimethylethyl)-chalcone,
2,6-dimethoxy-4'-di-methoxymethoxy-3-methyl-chalcone,
2,6-dimethoxy-4'-di-methoxymethoxy-3-ethyl-chalcone,
2,6-dimethoxy-4'-di-methoxymethoxy-3-propyl-chalcone,
2,6-dimethoxy-4'-di-methoxymethoxy-3-prop-2-enyl-chalcone,
2,6-dimethoxy-4'-di-methoxymethoxy-3-(1,1-dimethylprop-2-enyl)-chalcone,
2,6-dimethoxy-4'-di-methoxymethoxy-3-(1,1-dimethylethyl)-chalcone,
2,6-dimethoxy-4'-di-propenoxy-3-methyl-chalcone,
2,6-dimethoxy-4'-di-propenoxy-3-ethyl-chalcone,
2,6-dimethoxy-4'-di-propenoxy-3-propyl-chalcone,
2,6-dimethoxy-4'-di-propenoxy-3-prop-2-enyl-chalcone,
2,6-dimethoxy-4'-di-propenoxy-3-(1,1-dimethylprop-2-enyl)-chalcone, and
2,6-dimethoxy-4'-di-propenoxy-3-(1,1-dimethylethyl)-chalcone.

Specific examples of bis-aromatic α,β-unsaturated ketones are

2,6-dimethoxy-4'-(2-prop-2-enyloxy)-chalcone,
2,6-diethoxy-4'-(2-prop-2-enyloxy)-chalcone,
2,6-di-n-propoxy-4'-(2-prop-2-enyloxy)-chalcone,
2,6-diisopropoxy-4'-(2-prop-2-enyloxy)-chalcone,

2,6-di-n-butoxy-4'-(2-prop-2-enyloxy)-chalcone,
2,6-di-t-butoxy-4'-(2-prop-2-enyloxy)-chalcone,
2,6-dimethoxy-5-methyl-4'-(2-prop-2-enyloxy)-chalcone,
2,6-diethoxy-5-methyl-4'-(2-prop-2-enyloxy)-chalcone,
2,6-di-n-propoxy-5-methyl-4'-(2-prop-2-enyloxy)-chalcone,
2,6-diisopropoxy-5-methyl-4'-(2-prop-2-enyloxy)-chalcone,
2,6-di-n-butoxy-5-methyl-4'-(2-prop-2-enyloxy)-chalcone,
2,6-di-t-butoxy-5-methyl-4'-(2-prop-2-enyloxy)-chalcone,
2,6-dimethoxy-5-prop-2-enyl-4'-(2-prop-2-enyloxy)-chalcone,
2,6-diethoxy-5-prop-2-enyl-4'-(2-prop-2-enyloxy)-chalcone,
2,6-di-n-propoxy-5-prop-2-enyl-4'-(2-prop-2-enyloxy)-chalcone,
2,6-diisopropoxy-5-prop-2-enyl-4'-(2-prop-2-enyloxy)-chalcone,
2,6-di-n-butoxy-5-prop-2-enyl-4'-(2-prop-2-enyloxy)-chalcone,
2,6-di-t-butoxy-5-prop-2-enyl-4'-(2-prop-2-enyloxy)-chalcone,
2,6-dimethoxy-5-propyl-4'-(2-prop-2-enyloxy)-chalcone,
2,6-diethoxy-5-propyl-4'-(2-prop-2-enyloxy)-chalcone,
2,6-di-n-propoxy-5-propyl-4'-(2-prop-2-enyloxy)-chalcone,
2,6-diisopropoxy-5-propyl-4'-(2-prop-2-enyloxy)-chalcone,
2,6-di-n-butoxy-5-propyl-4'-(2-prop-2-enyloxy)-chalcone,
2,6-di-t-butoxy-5-propyl-4'-(2-prop-2-enyloxy)-chalcone,
2,6-dimethoxy-5-(1,1-dimethylethyl)-4'-(2-prop-2-enyloxy)-chalcone,
2,6-diethoxy-5-(1,1-dimethylethyl)-4'-(2-prop-2-enyloxy)-chalcone,
2,6-di-n-propoxy-5-(1,1-dimethylethyl)-4'-(2-prop-2-enyloxy)-chalcone,
2,6-diisopropoxy-5-(1,1-dimethylethyl)-4'-(2-prop-2-enyloxy)-chalcone,
2,6-di-n-butoxy-5-(1,1-dimethylethyl)-4'-(2-prop-2-enyloxy)-chalcone,
2,6-di-t-butoxy-5-(1,1-dimethylethyl)-4'-(2-prop-2-enyloxy)-chalcone,
2,6-dimethoxy-5-(1,1-dimethylethyl)-4'-(2-prop-2-enyloxy)-chalcone,
2,6-diethoxy-5-(1,1-dimethylethyl)-4'-(2-prop-2-enyloxy)-chalcone,
2,6-di-n-propoxy-5-(1,1-dimethylethyl)-4'-(2-prop-2-enyloxy)-chalcone,
2,6-diisopropoxy-5-(1,1-dimethylethyl)-4'-(2-prop-2-enyloxy)-chalcone,
2,6-di-n-butoxy-5-(1,1-dimethylethyl)-4'-(2-prop-2-enyloxy)-chalcone,
2,6-di-t-butoxy-5-(1,1-dimethylethyl)-4'-(2-prop-2-enyloxy)-chalcone.

2,5-dimethoxy-4'-methoxychalcone,
2,5-diethoxy-4'-methoxychalcone,
2,5-di-n-propoxy-4'-methoxychalcone,
2,5-diisopropoxy-4'-methoxychalcone,
2,5-di-n-butoxy-4'-methoxychalcone,
2,5-di-t-butoxy-4'-methoxychalcone,

2,5-dimethoxy-4'-ethoxychalcone

2,5-diethoxy-4'-ethoxychalcone,
2,5-di-n-propoxy-4'-ethoxychalcone,
2,5-diisopropoxy-4'-ethoxychalcone,
2,5-di-n-butoxy-4'-ethoxychalcone,
2,5-di-t-butoxy-4'-ethoxychalcone,

2,5-dimethoxy-4'-(2-prop-2-enyloxy)-chalcone,
2,5-diethoxy-4'-(2-prop-2-enyloxy)-chalcone,
2,5-di-n-propoxy-4'-(2-prop-2-enyloxy)-chalcone,
2,5-diisopropoxy-4'-(2-prop-2-enyloxy)-chalcone,
2,5-di-n-butoxy-4'-(2-prop-2-enyloxy)-chalcone,
2,5-di-t-butoxy-4'-(2-prop-2-enyloxy)-chalcone.

2-methoxy-4,4'-dihydroxy-5-methylchalcone
2-methoxy-4,4'-dihydroxy-5-ethylchalcone
2-methoxy-4,4'-dihydroxy-5-propylchalcone
2-methoxy-4,4'-dihydroxy-5-isopropylchalcone
2-methoxy-4,4'-dihydroxy-5-t-butylchalcone
2-methoxy-4,4'-dihydroxy-5-(1,1-dimethylpropyl)chalcone
2-methoxy-4,4'-dihydroxy-5-prop-2-enylchalcone
2-methoxy-4,4'-dihydroxy-5-but-2-enylchalcone
2-ethoxy-4,4'-dihydroxy-5-methylchalcone
2-ethoxy-4,4'-dihydroxy-5-ethylchalcone
2-ethoxy-4,4'-dihydroxy-5-propylchalcone
2-ethoxy-4,4'-dihydroxy-5-isopropylchalcone
2-ethoxy-4,4'-dihydroxy-5-t-butylchalcone
2-ethoxy-4,4'-dihydroxy-5-(1,1-dimethylpropyl)chalcone
2-ethoxy-4,4'-dihydroxy-5-prop-2-enylchalcone
2-ethoxy-4,4'-dihydroxy-5-but-2-enylchalcone
2-ethoxy-4,4'-dihydroxy-5-(1,1-dimethylprop-2-enyl)chalcone
2-propoxy-4,4'-dihydroxy-5-methylchalcone
2-propoxy-4,4'-dihydroxy-5-ethylchalcone
2-propoxy-4,4'-dihydroxy-5-propylchalcone
2-propoxy-4,4'-dihydroxy-5-isopropylchalcone
2-propoxy-4,4'-dihydroxy-5-t-butylchalcone
2-propoxy-4,4'-dihydroxy-5-(1,1-dimethylpropyl)chalcone
2-propoxy-4,4'-dihydroxy-5-prop-2-enylchalcone
2-propoxy-4,4'-dihydroxy-5-but-2-enylchalcone
2-propoxy-4,4'-dihydroxy-5-(1,1-dimethylprop-2-enyl)chalcone
2-isopropoxy-4,4'-dihydroxy-5-methylchalcone
2-isopropoxy-4,4'-dihydroxy-5-ethylchalcone

2-isopropoxy-4,4'-dihydroxy-5-propylchalcone
2-isopropoxy-4,4'-dihydroxy-5-isopropylchalcone
2-isopropoxy-4,4'-dihydroxy-5-t-butylchalcone
2-isopropoxy-4,4'-dihydroxy-5-(1,1-dimethylpropyl)chalcone
2-isopropoxy-4,4'-dihydroxy-5-prop-2-enylchalcone
2-isopropoxy-4,4'-dihydroxy-5-but-2-enylchalcone
2-isopropoxy-4,4'-dihydroxy-5-(1,1-dimethylprop-2-enyl)chalcone
2-methoxy-4-hydroxy-4'-amino-5-methylchalcone
2-methoxy-4-hydroxy-4'-amino-5-ethylchalcone
2-methoxy-4-hydroxy-4'-amino-5-propylchalcone
2-methoxy-4-hydroxy-4'-amino-5-isopropylchalcone
2-methoxy-4-hydroxy-4'-amino-5-t-butylchalcone
2-methoxy-4-hydroxy-4'-amino-5-(1,1-dimethylpropyl)chalcone
2-methoxy-4-hydroxy-4'-amino-5-prop-2-enylchalcone
2-methoxy-4-hydroxy-4'-amino-5-but-2-enylchalcone
2-ethoxy-4-hydroxy-4'-amino-5-methylchalcone
2-ethoxy-4-hydroxy-4'-amino-5-ethylchalcone
2-ethoxy-4-hydroxy-4'-amino-5-propylchalcone
2-ethoxy-4-hydroxy-4'-amino-5-isopropylchalcone
2-ethoxy-4-hydroxy-4'-amino-5-t-butylchalcone
2-ethoxy-4-hydroxy-4'-amino-5-(1,1-dimethylpropyl)chalcone
2-ethoxy-4-hydroxy-4'-amino-5-prop-2-enylchalcone
2-ethoxy-4-hydroxy-4'-amino-5-but-2-enylchalcone
2-ethoxy-4-hydroxy-4'-amino-5-(1,1-dimethylprop-2-enyl)chalcone
2-propoxy-4-hydroxy-4'-amino-5-methylchalcone
2-propoxy-4-hydroxy-4'-amino-5-ethylchalcone
2-propoxy-4-hydroxy-4'-amino-5-propylchalcone
2-propoxy-4-hydroxy-4'-amino-5-isopropylchalcone
2-propoxy-4-hydroxy-4'-amino-5-t-butylchalcone
2-propoxy-4-hydroxy-4'-amino-5-(1,1-dimethylpropyl)chalcone
2-propoxy-4-hydroxy-4'-amino-5-prop-2-enylchalcone
2-propoxy-4-hydroxy-4'-amino-5-but-2-enylchalcone
2-propoxy-4-hydroxy-4'-amino-5-(1,1-dimethylprop-2-enyl)chalcone
2-isopropoxy-4-hydroxy-4'-amino-5-methylchalcone
2-isopropoxy-4-hydroxy-4'-amino-5-ethylchalcone
2-isopropoxy-4-hydroxy-4'-amino-5-propylchalcone
2-isopropoxy-4-hydroxy-4'-amino-5-isopropylchalcone
2-isopropoxy-4-hydroxy-4'-amino-5-t-butylchalcone
2-isopropoxy-4-hydroxy-4'-amino-5-(1,1-dimethylpropyl)chalcone
2-isopropoxy-4-hydroxy-4'-amino-5-prop-2-enylchalcone
2-isopropoxy-4-hydroxy-4'-amino-5-but-2-enylchalcone

2-isopropoxy-4-hydroxy-4'-amino-5-(1,1-dimethylprop-2-enyl)chalcone
2-methoxy-4-hydroxy-4'-methylamino-5-methylchalcone
2-methoxy-4-hydroxy-4'-methylamino-5-ethylchalcone
2-methoxy-4-hydroxy-4'-methylamino-5-propylchalcone
2-methoxy-4-hydroxy-4'-methylamino-5-isopropylchalcone
2-methoxy-4-hydroxy-4'-methylamino-5-t-butylchalcone
2-methoxy-4-hydroxy-4'-methylamino-5-(1,1-dimethylpropyl)chalcone
2-methoxy-4-hydroxy-4'-methylamino-5-prop-2-enylchalcone
2-methoxy-4-hydroxy-4'-methylamino-5-but-2-enylchalcone
2-ethoxy-4-hydroxy-4'-methylamino-5-methylchalcone
2-ethoxy-4-hydroxy-4'-methylamino-5-ethylchalcone
2-ethoxy-4-hydroxy-4'-methylamino-5-propylchalcone
2-ethoxy-4-hydroxy-4'-methylamino-5-isopropylchalcone
2-ethoxy-4-hydroxy-4'-methylamino-5-t-butylchalcone
2-ethoxy-4-hydroxy-4'-methylamino-5-(1,1-dimethylpropyl)chalcone
2-ethoxy-4-hydroxy-4'-methylamino-5-prop-2-enylchalcone
2-ethoxy-4-hydroxy-4'-methylamino-5-but-2-enylchalcone
2-ethoxy-4-hydroxy-4'-methylamino-5-(1,1-dimethylprop-2-enyl)chalcone
2-propoxy-4-hydroxy-4'-methylamino-5-methylchalcone
2-propoxy-4-hydroxy-4'-methylamino-5-ethylchalcone
2-propoxy-4-hydroxy-4'-methylamino-5-propylchalcone
2-propoxy-4-hydroxy-4'-methylamino-5-isopropylchalcone
2-propoxy-4-hydroxy-4'-methylamino-5-t-butylchalcone
2-propoxy-4-hydroxy-4'-methylamino-5-(1,1-dimethylpropyl)chalcone
2-propoxy-4-hydroxy-4'-methylamino-5-prop-2-enylchalcone
2-propoxy-4-hydroxy-4'-methylamino-5-but-2-enylchalcone
2-propoxy-4-hydroxy-4'-methylamino-5-(1,1-dimethylprop-2-enyl)chalcone
2-isopropoxy-4-hydroxy-4'-methylamino-5-methylchalcone
2-isopropoxy-4-hydroxy-4'-methylamino-5-ethylchalcone
2-isopropoxy-4-hydroxy-4'-methylamino-5-propylchalcone
2-isopropoxy-4-hydroxy-4'-methylamino-5-isopropylchalcone
2-isopropoxy-4-hydroxy-4'-methylamino-5-t-butylchalcone
2-isopropoxy-4-hydroxy-4'-methylamino-5-(1,1-dimethylpropyl)chalcone
2-isopropoxy-4-hydroxy-4'-methylamino-5-prop-2-enylchalcone
2-isopropoxy-4-hydroxy-4'-methylamino-5-but-2-enylchalcone
2-isopropoxy-4-hydroxy-4'-methylamino-5-(1,1-dimethylprop-2-enyl)chalcone

and the corresponding ketones in which Z is selected from the groups (A)-(E) as defined above

2-methoxy-4,4'-dipivaloyloxy-5-methylchalcone

2-methoxy-4,4'-dipivaloyloxy-5-ethylchalcone
2-methoxy-4,4'-dipivaloyloxy-5-propylchalcone
2-methoxy-4,4'-dipivaloyloxy-5-isopropylchalcone
2-methoxy-4,4'-dipivaloyloxy-5-t-butylchalcone
2-methoxy-4,4'-dipivaloyloxy-5-(1,1-dimethylpropyl)chalcone
2-methoxy-4,4'-dipivaloyloxy-5-prop-2-enylchalcone
2-methoxy-4,4'-dipivaloyloxy-5-but-2-enylchalcone
2-ethoxy-4,4'-dipivaloyloxy-5-methylchalcone
2-ethoxy-4,4'-dipivaloyloxy-5-ethylchalcone
2-ethoxy-4,4'-dipivaloyloxy-5-propylchalcone
2-ethoxy-4,4'-dipivaloyloxy-5-isopropylchalcone
2-ethoxy-4,4'-dipivaloyloxy-5-t-butylchalcone
2-ethoxy-4,4'-dipivaloyloxy-5-(1,1-dimethylpropyl)chalcone
2-ethoxy-4,4'-dipivaloyloxy-5-prop-2-enylchalcone
2-ethoxy-4,4'-dipivaloyloxy-5-but-2-enylchalcone
2-ethoxy-4,4'-dipivaloyloxy-5-(1,1-dimethylprop-2-enyl)chalcone
2-propoxy-4,4'-dipivaloyloxy-5-methylchalcone
2-propoxy-4,4'-dipivaloyloxy-5-ethylchalcone
2-propoxy-4,4'-dipivaloyloxy-5-propylchalcone
2-propoxy-4,4'-dipivaloyloxy-5-isopropylchalcone
2-propoxy-4,4'-dipivaloyloxy-5-t-butylchalcone
2-propoxy-4,4'-dipivaloyloxy-5-(1,1-dimethylpropyl)chalcone
2-propoxy-4,4'-dipivaloyloxy-5-prop-2-enylchalcone
2-propoxy-4,4'-dipivaloyloxy-5-but-2-enylchalcone
2-propoxy-4,4'-dipivaloyloxy-5-(1,1-dimethylprop-2-enyl)chalcone
2-isopropoxy-4,4'-dipivaloyloxy-5-methylchalcone
2-isopropoxy-4,4'-dipivaloyloxy-5-ethylchalcone
2-isopropoxy-4,4'-dipivaloyloxy-5-propylchalcone
2-isopropoxy-4,4'-dipivaloyloxy-5-isopropylchalcone
2-isopropoxy-4,4'-dipivaloyloxy-5-t-butylchalcone
2-isopropoxy-4,4'-dipivaloyloxy-5-(1,1-dimethylpropyl)chalcone
2-isopropoxy-4,4'-dipivaloyloxy-5-prop-2-enylchalcone
2-isopropoxy-4,4'-dipivaloyloxy-5-but-2-enylchalcone
2-isopropoxy-4,4'-dipivaloyloxy-5-(1,1-dimethylprop-2-enyl)chalcone
2-methoxy-4,4'-dipivaloyloxymethoxy-5-methylchalcone
2-methoxy-4,4'-dipivaloyloxymethoxy-5-ethylchalcone
2-methoxy-4,4'-dipivaloyloxymethoxy-5-propylchalcone
2-methoxy-4,4'-dipivaloyloxymethoxy-5-isopropylchalcone
2-methoxy-4,4'-dipivaloyloxymethoxy-5-t-butylchalcone
2-methoxy-4,4'-dipivaloyloxymethoxy-5-(1,1-dimethylpropyl)chalcone
2-methoxy-4,4'-dipivaloyloxymethoxy-5-prop-2-enylchalcone

2-methoxy-4,4'-dipivaloyloxymethoxy-5-but-2-enylchalcone
2-ethoxy-4,4'-dipivaloyloxymethoxy-5-methylchalcone
2-ethoxy-4,4'-dipivaloyloxymethoxy-5-ethylchalcone
2-ethoxy-4,4'-dipivaloyloxymethoxy-5-propylchalcone
2-ethoxy-4,4'-dipivaloyloxymethoxy-5-isopropylchalcone
2-ethoxy-4,4'-dipivaloyloxymethoxy-5-t-butylchalcone
2-ethoxy-4,4'-dipivaloyloxymethoxy-5-(1,1-dimethylpropyl)chalcone
2-ethoxy-4,4'-dipivaloyloxymethoxy-5-prop-2-enylchalcone
2-ethoxy-4,4'-dipivaloyloxymethoxy-5-but-2-enylchalcone
2-ethoxy-4,4'-dipivaloyloxymethoxy-5-(1,1-dimethylprop-2-enyl)chalcone
2-propoxy-4,4'-dipivaloyloxymethoxy-5-methylchalcone
2-propoxy-4,4'-dipivaloyloxymethoxy-5-ethylchalcone
2-propoxy-4,4'-dipivaloyloxymethoxy-5-propylchalcone
2-propoxy-4,4'-dipivaloyloxymethoxy-5-isopropylchalcone
2-propoxy-4,4'-dipivaloyloxymethoxy-5-t-butylchalcone
2-propoxy-4,4'-dipivaloyloxymethoxy-5-(1,1-dimethylpropyl)chalcone
2-propoxy-4,4'-dipivaloyloxymethoxy-5-prop-2-enylchalcone
2-propoxy-4,4'-dipivaloyloxymethoxy-5-but-2-enylchalcone
2-propoxy-4,4'-dipivaloyloxymethoxy-5-(1,1-dimethylprop-2-enyl)chalcone
2-isopropoxy-4,4'-dipivaloyloxymethoxy-5-methylchalcone
2-isopropoxy-4,4'-dipivaloyloxymethoxy-5-ethylchalcone
2-isopropoxy-4,4'-dipivaloyloxymethoxy-5-propylchalcone
2-isopropoxy-4,4'-dipivaloyloxymethoxy-5-isopropylchalcone
2-isopropoxy-4,4'-dipivaloyloxymethoxy-5-t-butylchalcone
2-isopropoxy-4,4'-dipivaloyloxymethoxy-5-(1,1-dimethylpropyl)chalcone
2-isopropoxy-4,4'-dipivaloyloxymethoxy-5-prop-2-enylchalcone
2-isopropoxy-4,4'-dipivaloyloxymethoxy-5-but-2-enylchalcone
2-isopropoxy-4,4'-dipivaloyloxymethoxy-5-(1,1-dimethylprop-2-enyl)chalcone
2-methoxy-4,4'-(N,N-dimethylcarbamoyl)-5-methylchalcone
2-methoxy-4,4'-(N,N-dimethylcarbamoyl)-5-ethylchalcone
2-methoxy-4,4'-(N,N-dimethylcarbamoyl)-5-propylchalcone
2-methoxy-4,4'-(N,N-dimethylcarbamoyl)-5-isopropylchalcone
2-methoxy-4,4'-(N,N-dimethylcarbamoyl)-5-t-butylchalcone
2-methoxy-4,4'-(N,N-dimethylcarbamoyl)-5-(1,1-dimethylpropyl)chalcone
2-methoxy-4,4'-(N,N-dimethylcarbamoyl)-5-prop-2-enylchalcone
2-methoxy-4,4'-(N,N-dimethylcarbamoyl)-5-but-2-enylchalcone
2-ethoxy-4,4'-(N,N-dimethylcarbamoyl)-5-methylchalcone
2-ethoxy-4,4'-(N,N-dimethylcarbamoyl)-5-ethylchalcone
2-ethoxy-4,4'-(N,N-dimethylcarbamoyl)-5-propylchalcone
2-ethoxy-4,4'-(N,N-dimethylcarbamoyl)-5-isopropylchalcone
2-ethoxy-4,4'-(N,N-dimethylcarbamoyl)-5-t-butylchalcone

2-ethoxy-4,4'-(N,N-dimethylcarbamoyl)-5-(1,1-dimethylpropyl)chalcone
2-ethoxy-4,4'-(N,N-dimethylcarbamoyl)-5-prop-2-enylchalcone
2-ethoxy-4,4'-(N,N-dimethylcarbamoyl)-5-but-2-enylchalcone
2-ethoxy-4,4'-(N,N-dimethylcarbamoyl)-5-(1,1-dimethylprop-2-enyl)chalcone
2-propoxy-4,4'-(N,N-dimethylcarbamoyl)-5-methylchalcone
2-propoxy-4,4'-(N,N-dimethylcarbamoyl)-5-ethylchalcone
2-propoxy-4,4'-(N,N-dimethylcarbamoyl)-5-propylchalcone
2-propoxy-4,4'-(N,N-dimethylcarbamoyl)-5-isopropylchalcone
2-propoxy-4,4'-(N,N-dimethylcarbamoyl)-5-t-butylchalcone
2-propoxy-4,4'-(N,N-dimethylcarbamoyl)-5-(1,1-dimethylpropyl)chalcone
2-propoxy-4,4'-(N,N-dimethylcarbamoyl)-5-prop-2-enylchalcone
2-propoxy-4,4'-(N,N-dimethylcarbamoyl)-5-but-2-enylchalcone
2-propoxy-4,4'-(N,N-dimethylcarbamoyl)-5-(1,1-dimethylprop-2-enyl)chalcone
2-isopropoxy-4,4'-(N,N-dimethylcarbamoyl)-5-methylchalcone
2-isopropoxy-4,4'-(N,N-dimethylcarbamoyl)-5-ethylchalcone
2-isopropoxy-4,4'-(N,N-dimethylcarbamoyl)-5-propylchalcone
2-isopropoxy-4,4'-(N,N-dimethylcarbamoyl)-5-isopropylchalcone
2-isopropoxy-4,4'-(N,N-dimethylcarbamoyl)-5-t-butylchalcone
2-isopropoxy-4,4'-(N,N-dimethylcarbamoyl)-5-(1,1-dimethylpropyl)chalcone
2-isopropoxy-4,4'-(N,N-dimethylcarbamoyl)-5-prop-2-enylchalcone
2-isopropoxy-4,4'-(N,N-dimethylcarbamoyl)-5-but-2-enylchalcone
2-isopropoxy-4,4'-(N,N-dimethylcarbamoyl)-5-(1,1-dimethylprop-2-enyl)chalcone

Specific examples of bis-aromatic α,β-unsaturated ketones are

2-methoxy-4,4'-dihydroxy-5-methylchalcone
2-methoxy-4,4'-dihydroxy-3,5-diethylchalcone
2-methoxy-4,4'-dihydroxy-3,5-dipropylchalcone
2-methoxy-4,4'-dihydroxy-3,5-diisopropylchalcone
2-methoxy-4,4'-dihydroxy-3,5-di-t-butylchalcone
2-methoxy-4,4'-dihydroxy-3,5-di-(1,1-dimethylpropyl)chalcone
2-methoxy-4,4'-dihydroxy-3,5-diprop-2-enylchalcone
2-methoxy-4,4'-dihydroxy-3,5-dibut-2-enylchalcone
2-ethoxy-4,4'-dihydroxy-3,5-dimethylchalcone
2-ethoxy-4,4'-dihydroxy-3,5-diethylchalcone
2-ethoxy-4,4'-dihydroxy-3,5-dipropylchalcone
2-ethoxy-4,4'-dihydroxy-3,5-diisopropylchalcone
2-ethoxy-4,4'-dihydroxy-3,5-di-t-butylchalcone
2-ethoxy-4,4'-dihydroxy-3,5-di-(1,1-dimethylpropyl)chalcone
2-ethoxy-4,4'-dihydroxy-3,5-diprop-2-enylchalcone
2-ethoxy-4,4'-dihydroxy-3,5-dibut-2-enylchalcone

2-ethoxy-4,4'-dihydroxy-3,5-di-(1,1-dimethylprop-2-enyl)chalcone
2-propoxy-4,4'-dihydroxy-3,5-dimethylchalcone
2-propoxy-4,4'-dihydroxy-3,5-diethylchalcone
2-propoxy-4,4'-dihydroxy-3,5-dipropylchalcone
2-propoxy-4,4'-dihydroxy-3,5-diisopropylchalcone
2-propoxy-4,4'-dihydroxy-3,5-di-t-butylchalcone
2-propoxy-4,4'-dihydroxy-3,5-di-(1,1-dimethylpropyl)chalcone
2-propoxy-4,4'-dihydroxy-3,5-diprop-2-enylchalcone
2-propoxy-4,4'-dihydroxy-3,5-dibut-2-enylchalcone
2-propoxy-4,4'-dihydroxy-3,5-di-(1,1-dimethylprop-2-enyl)chalcone
2-isopropoxy-4,4'-dihydroxy-3,5-dimethylchalcone
2-isopropoxy-4,4'-dihydroxy-3,5-diethylchalcone
2-isopropoxy-4,4'-dihydroxy-3,5-dipropylchalcone
2-isopropoxy-4,4'-dihydroxy-3,5-diisopropylchalcone
2-isopropoxy-4,4'-dihydroxy-3,5-di-t-butylchalcone
2-isopropoxy-4,4'-dihydroxy-3,5-di-(1,1-dimethylpropyl)chalcone
2-isopropoxy-4,4'-dihydroxy-3,5-diprop-2-enylchalcone
2-isopropoxy-4,4'-dihydroxy-3,5-dibut-2-enylchalcone
2-isopropoxy-4,4'-dihydroxy-3,5-di-(1,1-dimethylprop-2-enyl)chalcone
2-methoxy-4-hydroxy-4'-amino-3,5-dimethylchalcone
2-methoxy-4-hydroxy-4'-amino-3,5-diethylchalcone
2-methoxy-4-hydroxy-4'-amino-3,5-dipropylchalcone
2-methoxy-4-hydroxy-4'-amino-3,5-diisopropylchalcone
2-methoxy-4-hydroxy-4'-amino-3,5-di-t-butylchalcone
2-methoxy-4-hydroxy-4'-amino-3,5-di-(1,1-dimethylpropyl)chalcone
2-methoxy-4-hydroxy-4'-amino-3,5-diprop-2-enylchalcone
2-methoxy-4-hydroxy-4'-amino-3,5-dibut-2-enylchalcone
2-ethoxy-4-hydroxy-4'-amino-3,5-dimethylchalcone
2-ethoxy-4-hydroxy-4'-amino-3,5-diethylchalcone
2-ethoxy-4-hydroxy-4'-amino-3,5-dipropylchalcone
2-ethoxy-4-hydroxy-4'-amino-3,5-diisopropylchalcone
2-ethoxy-4-hydroxy-4'-amino-3,5-di-t-butylchalcone
2-ethoxy-4-hydroxy-4'-amino-3,5-di-(1,1-dimethylpropyl)chalcone
2-ethoxy-4-hydroxy-4'-amino-3,5-diprop-2-enylchalcone
2-ethoxy-4-hydroxy-4'-amino-3,5-dibut-2-enylchalcone
2-ethoxy-4-hydroxy-4'-amino-3,5-di-(1,1-dimethylprop-2-enyl)chalcone
2-propoxy-4-hydroxy-4'-amino-3,5-dimethylchalcone
2-propoxy-4-hydroxy-4'-amino-3,5-diethylchalcone
2-propoxy-4-hydroxy-4'-amino-3,5-dipropylchalcone
2-propoxy-4-hydroxy-4'-amino-3,5-diisopropylchalcone
2-propoxy-4-hydroxy-4'-amino-3,5-di-t-butylchalcone

2-propoxy-4-hydroxy-4'-amino-3,5-di-(1,1-dimethylpropyl)chalcone
2-propoxy-4-hydroxy-4'-amino-3,5-diprop-2-enylchalcone
2-propoxy-4-hydroxy-4'-amino-3,5-dibut-2-enylchalcone
2-propoxy-4-hydroxy-4'-amino-3,5-di-(1,1-dimethylprop-2-enyl)chalcone
2-isopropoxy-4-hydroxy-4'-amino-3,5-dimethylchalcone
2-isopropoxy-4-hydroxy-4'-amino-3,5-diethylchalcone
2-isopropoxy-4-hydroxy-4'-amino-3,5-dipropylchalcone
2-isopropoxy-4-hydroxy-4'-amino-3,5-diisopropylchalcone
2-isopropoxy-4-hydroxy-4'-amino-3,5-di-t-butylchalcone
2-isopropoxy-4-hydroxy-4'-amino-3,5-di-(1,1-dimethylpropyl)chalcone
2-isopropoxy-4-hydroxy-4'-amino-3,5-diprop-2-enylchalcone
2-isopropoxy-4-hydroxy-4'-amino-3,5-dibut-2-enylchalcone
2-isopropoxy-4-hydroxy-4'-amino-5-(1,1-dimethylprop-2-enyl)chalcone
2-methoxy-4-hydroxy-4'-methylamino-3,5-dimethylchalcone
2-methoxy-4-hydroxy-4'-methylamino-3,5-diethylchalcone
2-methoxy-4-hydroxy-4'-methylamino-3,5-dipropylchalcone
2-methoxy-4-hydroxy-4'-methylamino-3,5-diisopropylchalcone
2-methoxy-4-hydroxy-4'-methylamino-3,5-di-t-butylchalcone
2-methoxy-4-hydroxy-4'-methylamino-3,5-di-(1,1-dimethylpropyl)chalcone
2-methoxy-4-hydroxy-4'-methylamino-3,5-diprop-2-enylchalcone
2-methoxy-4-hydroxy-4'-methylamino-3,5-dibut-2-enylchalcone
2-ethoxy-4-hydroxy-4'-methylamino-3,5-dimethylchalcone
2-ethoxy-4-hydroxy-4'-methylamino-3,5-diethylchalcone
2-ethoxy-4-hydroxy-4'-methylamino-3,5-dipropylchalcone
2-ethoxy-4-hydroxy-4'-methylamino-3,5-diisopropylchalcone
2-ethoxy-4-hydroxy-4'-methylamino-3,5-di-t-butylchalcone
2-ethoxy-4-hydroxy-4'-methylamino-3,5-di-(1,1-dimethylpropyl)chalcone
2-ethoxy-4-hydroxy-4'-methylamino-3,5-diprop-2-enylchalcone
2-ethoxy-4-hydroxy-4'-methylamino-3,5-dibut-2-enylchalcone
2-ethoxy-4-hydroxy-4'-methylamino-5-(1,1-dimethylprop-2-enyl)chalcone
2-propoxy-4-hydroxy-4'-methylamino-3,5-dimethylchalcone
2-propoxy-4-hydroxy-4'-methylamino-3,5-diethylchalcone
2-propoxy-4-hydroxy-4'-methylamino-3,5-dipropylchalcone
2-propoxy-4-hydroxy-4'-methylamino-3,5-diisopropylchalcone
2-propoxy-4-hydroxy-4'-methylamino-3,5-di-t-butylchalcone
2-propoxy-4-hydroxy-4'-methylamino-3,5-di-(1,1-dimethylpropyl)chalcone
2-propoxy-4-hydroxy-4'-methylamino-3,5-diprop-2-enylchalcone
2-propoxy-4-hydroxy-4'-methylamino-3,5-dibut-2-enylchalcone
2-propoxy-4-hydroxy-4'-methylamino-5-(1,1-dimethylprop-2-enyl)chalcone
2-isopropoxy-4-hydroxy-4'-methylamino-3,5-dimethylchalcone
2-isopropoxy-4-hydroxy-4'-methylamino-3,5-diethylchalcone

2-isopropoxy-4-hydroxy-4'-methylamino-3,5-dipropylchalcone
2-isopropoxy-4-hydroxy-4'-methylamino-3,5-diisopropylchalcone
2-isopropoxy-4-hydroxy-4'-methylamino-3,5-di-t-butylchalcone
2-isopropoxy-4-hydroxy-4'-methylamino-3,5-di-(1,1-dimethylpropyl)chalcone
2-isopropoxy-4-hydroxy-4'-methylamino-3,5-diprop-2-enylchalcone
2-isopropoxy-4-hydroxy-4'-methylamino-3,5-dibut-2-enylchalcone
2-isopropoxy-4-hydroxy-4'-methylamino-5-(1,1-dimethylprop-2-enyl)chalcone

and the corresponding ketones in which Z is selected form the groups (A)-(E) as defined above

2-methoxy-4,4'-dipivaloyloxy-3,5-dimethylchalcone
2-methoxy-4,4'-dipivaloyloxy-3,5-diethylchalcone
2-methoxy-4,4'-dipivaloyloxy-3,5-dipropylchalcone
2-methoxy-4,4'-dipivaloyloxy-3,5-diisopropylchalcone
2-methoxy-4,4'-dipivaloyloxy-3,5-di-t-butylchalcone
2-methoxy-4,4'-dipivaloyloxy-3,5-di-(1,1-dimethylpropyl)chalcone
2-methoxy-4,4'-dipivaloyloxy-3,5-diprop-2-enylchalcone
2-methoxy-4,4'-dipivaloyloxy-3,5-dibut-2-enylchalcone
2-ethoxy-4,4'-dipivaloyloxy-3,5-dimethylchalcone
2-ethoxy-4,4'-dipivaloyloxy-3,5-diethylchalcone
2-ethoxy-4,4'-dipivaloyloxy-3,5-dipropylchalcone
2-ethoxy-4,4'-dipivaloyloxy-3,5-diisopropylchalcone
2-ethoxy-4,4'-dipivaloyloxy-3,5-di-t-butylchalcone
2-ethoxy-4,4'-dipivaloyloxy-3,5-di-(1,1-dimethylpropyl)chalcone
2-ethoxy-4,4'-dipivaloyloxy-3,5-diprop-2-enylchalcone
2-ethoxy-4,4'-dipivaloyloxy-3,5-dibut-2-enylchalcone
2-ethoxy-4,4'-dipivaloyloxy-3,5-di-(1,1-dimethylprop-2-enyl)chalcone
2-propoxy-4,4'-dipivaloyloxy-3,5-dimethylchalcone
2-propoxy-4,4'-dipivaloyloxy-3,5-diethylchalcone
2-propoxy-4,4'-dipivaloyloxy-3,5-dipropylchalcone
2-propoxy-4,4'-dipivaloyloxy-3,5-diisopropylchalcone
2-propoxy-4,4'-dipivaloyloxy-3,5-di-t-butylchalcone
2-propoxy-4,4'-dipivaloyloxy-3,5-di-(1,1-dimethylpropyl)chalcone
2-propoxy-4,4'-dipivaloyloxy-3,5-diprop-2-enylchalcone
2-propoxy-4,4'-dipivaloyloxy-3,5-dibut-2-enylchalcone
2-propoxy-4,4'-dipivaloyloxy-3,5-di-(1,1-dimethylprop-2-enyl)chalcone
2-isopropoxy-4,4'-dipivaloyloxy-3,5-dimethylchalcone
2-isopropoxy-4,4'-dipivaloyloxy-3,5-diethylchalcone
2-isopropoxy-4,4'-dipivaloyloxy-3,5-dipropylchalcone
2-isopropoxy-4,4'-dipivaloyloxy-3,5-diisopropylchalcone

2-isopropoxy-4,4'-dipivaloyloxy-3,5-di-t-butylchalcone
2-isopropoxy-4,4'-dipivaloyloxy-3,5-di-(1,1-dimethylpropyl)chalcone
2-isopropoxy-4,4'-dipivaloyloxy-3,5-diprop-2-enylchalcone
2-isopropoxy-4,4'-dipivaloyloxy-3,5-dibut-2-enylchalcone
2-isopropoxy-4,4'-dipivaloyloxy-3,5-di-(1,1-dimethylprop-2-enyl)chalcone
2-methoxy-4,4'-dipivaloyloxymethoxy-3,5-dimethylchalcone
2-methoxy-4,4'-dipivaloyloxymethoxy-3,5-diethylchalcone
2-methoxy-4,4'-dipivaloyloxymethoxy-3,5-dipropylchalcone
2-methoxy-4,4'-dipivaloyloxymethoxy-3,5-diisopropylchalcone
2-methoxy-4,4'-dipivaloyloxymethoxy-3,5-di-t-butylchalcone
2-methoxy-4,4'-dipivaloyloxymethoxy-3,5-di-(1,1-dimethylpropyl)chalcone
2-methoxy-4,4'-dipivaloyloxymethoxy-3,5-diprop-2-enylchalcone
2-methoxy-4,4'-dipivaloyloxymethoxy-3,5-dibut-2-enylchalcone
2-ethoxy-4,4'-dipivaloyloxymethoxy-3,5-dimethylchalcone
2-ethoxy-4,4'-dipivaloyloxymethoxy-3,5-diethylchalcone
2-ethoxy-4,4'-dipivaloyloxymethoxy-3,5-dipropylchalcone
2-ethoxy-4,4'-dipivaloyloxymethoxy-3,5-diisopropylchalcone
2-ethoxy-4,4'-dipivaloyloxymethoxy-3,5-di-t-butylchalcone
2-ethoxy-4,4'-dipivaloyloxymethoxy-3,5-di-(1,1-dimethylpropyl)chalcone
2-ethoxy-4,4'-dipivaloyloxymethoxy-3,5-diprop-2-enylchalcone
2-ethoxy-4,4'-dipivaloyloxymethoxy-3,5-dibut-2-enylchalcone
2-ethoxy-4,4'-dipivaloyloxymethoxy-5-(1,1-dimethylprop-2-enyl)chalcone
2-propoxy-4,4'-dipivaloyloxymethoxy-3,5-dimethylchalcone
2-propoxy-4,4'-dipivaloyloxymethoxy-3,5-diethylchalcone
2-propoxy-4,4'-dipivaloyloxymethoxy-3,5-dipropylchalcone
2-propoxy-4,4'-dipivaloyloxymethoxy-3,5-diisopropylchalcone
2-propoxy-4,4'-dipivaloyloxymethoxy-3,5-di-t-butylchalcone
2-propoxy-4,4'-dipivaloyloxymethoxy-3,5-di-(1,1-dimethylpropyl)chalcone
2-propoxy-4,4'-dipivaloyloxymethoxy-3,5-diprop-2-enylchalcone
2-propoxy-4,4'-dipivaloyloxymethoxy-3,5-dibut-2-enylchalcone
2-propoxy-4,4'-dipivaloyloxymethoxy-5-(1,1-dimethylprop-2-enyl)chalcone
2-isopropoxy-4,4'-dipivaloyloxymethoxy-3,5-dimethylchalcone
2-isopropoxy-4,4'-dipivaloyloxymethoxy-3,5-diethylchalcone
2-isopropoxy-4,4'-dipivaloyloxymethoxy-3,5-dipropylchalcone
2-isopropoxy-4,4'-dipivaloyloxymethoxy-3,5-diisopropylchalcone
2-isopropoxy-4,4'-dipivaloyloxymethoxy-3,5-di-t-butylchalcone
2-isopropoxy-4,4'-dipivaloyloxymethoxy-3,5-di-(1,1-dimethylpropyl)chalcone
2-isopropoxy-4,4'-dipivaloyloxymethoxy-3,5-diprop-2-enylchalcone
2-isopropoxy-4,4'-dipivaloyloxymethoxy-3,5-dibut-2-enylchalcone
2-isopropoxy-4,4'-dipivaloyloxymethoxy-5-(1,1-dimethylprop-2-enyl)chalcone
2-methoxy-4,4'-(N,N-dimethylcarbamoyl)-3,5-dimethylchalcone

2-methoxy-4,4'-(N,N-dimethylcarbamoyl)-3,5-diethylchalcone
2-methoxy-4,4'-(N,N-dimethylcarbamoyl)-3,5-dipropylchalcone
2-methoxy-4,4'-(N,N-dimethylcarbamoyl)-3,5-diisopropylchalcone
2-methoxy-4,4'-(N,N-dimethylcarbamoyl)-3,5-di-t-butylchalcone
2-methoxy-4,4'-(N,N-dimethylcarbamoyl)-5-(1,1-dimethylpropyl)chalcone
2-methoxy-4,4'-(N,N-dimethylcarbamoyl)-3,5-diprop-2-enylchalcone
2-methoxy-4,4'-(N,N-dimethylcarbamoyl)-3,5-dibut-2-enylchalcone
2-ethoxy-4,4'-(N,N-dimethylcarbamoyl)-3,5-dimethylchalcone
2-ethoxy-4,4'-(N,N-dimethylcarbamoyl)-3,5-diethylchalcone
2-ethoxy-4,4'-(N,N-dimethylcarbamoyl)-3,5-dipropylchalcone
2-ethoxy-4,4'-(N,N-dimethylcarbamoyl)-3,5-diisopropylchalcone
2-ethoxy-4,4'-(N,N-dimethylcarbamoyl)-3,5-di-t-butylchalcone
2-ethoxy-4,4'-(N,N-dimethylcarbamoyl)-5-(1,1-dimethylpropyl)chalcone
2-ethoxy-4,4'-(N,N-dimethylcarbamoyl)-3,5-diprop-2-enylchalcone
2-ethoxy-4,4'-(N,N-dimethylcarbamoyl)-3,5-dibut-2-enylchalcone
2-ethoxy-4,4'-(N,N-dimethylcarbamoyl)-5-(1,1-dimethylprop-2-enyl)chalcone
2-propoxy-4,4'-(N,N-dimethylcarbamoyl)-3,5-dimethylchalcone
2-propoxy-4,4'-(N,N-dimethylcarbamoyl)-3,5-diethylchalcone
2-propoxy-4,4'-(N,N-dimethylcarbamoyl)-3,5-dipropylchalcone
2-propoxy-4,4'-(N,N-dimethylcarbamoyl)-3,5-diisopropylchalcone
2-propoxy-4,4'-(N,N-dimethylcarbamoyl)-3,5-di-t-butylchalcone
2-propoxy-4,4'-(N,N-dimethylcarbamoyl)-5-(1,1-dimethylpropyl)chalcone
2-propoxy-4,4'-(N,N-dimethylcarbamoyl)-3,5-diprop-2-enylchalcone
2-propoxy-4,4'-(N,N-dimethylcarbamoyl)-3,5-dibut-2-enylchalcone
2-propoxy-4,4'-(N,N-dimethylcarbamoyl)-5-(1,1-dimethylprop-2-enyl)chalcone
2-isopropoxy-4,4'-(N,N-dimethylcarbamoyl)-3,5-dimethylchalcone
2-isopropoxy-4,4'-(N,N-dimethylcarbamoyl)-3,5-diethylchalcone
2-isopropoxy-4,4'-(N,N-dimethylcarbamoyl)-3,5-dipropylchalcone
2-isopropoxy-4,4'-(N,N-dimethylcarbamoyl)-3,5-diisopropylchalcone
2-isopropoxy-4,4'-(N,N-dimethylcarbamoyl)-3,5-di-t-butylchalcone
2-isopropoxy-4,4'-(N,N-dimethylcarbamoyl)-5-(1,1-dimethylpropyl)chalcone
2-isopropoxy-4,4'-(N,N-dimethylcarbamoyl)-3,5-diprop-2-enylchalcone
2-isopropoxy-4,4'-(N,N-dimethylcarbamoyl)-3,5-dibut-2-enylchalcone
2-isopropoxy-4,4'-(N,N-dimethylcarbamoyl)-5-(1,1-dimethylprop-2-enyl)chalcone

Specific examples of bis-aromatic α,β-unsaturated ketones are

2-methoxy-6,4'-dihydroxy-5-methylchalcone
2-methoxy-6,4'-dihydroxy-3,5-diethylchalcone
2-methoxy-6,4'-dihydroxy-3,5-dipropylchalcone
2-methoxy-6,4'-dihydroxy-3,5-diisopropylchalcone

2-methoxy-6,4'-dihydroxy-3,5-di-t-butylchalcone
2-methoxy-6,4'-dihydroxy-3,5-di-(1,1-dimethylpropyl)chalcone
2-methoxy-6,4'-dihydroxy-3,5-diprop-2-enylchalcone
2-methoxy-6,4'-dihydroxy-3,5-dibut-2-enylchalcone
2-ethoxy-6,4'-dihydroxy-3,5-dimethylchalcone
2-ethoxy-6,4'-dihydroxy-3,5-diethylchalcone
2-ethoxy-6,4'-dihydroxy-3,5-dipropylchalcone
2-ethoxy-6,4'-dihydroxy-3,5-diisopropylchalcone
2-ethoxy-6,4'-dihydroxy-3,5-di-t-butylchalcone
2-ethoxy-6,4'-dihydroxy-3,5-di-(1,1-dimethylpropyl)chalcone
2-ethoxy-6,4'-dihydroxy-3,5-diprop-2-enylchalcone
2-ethoxy-6,4'-dihydroxy-3,5-dibut-2-enylchalcone
2-ethoxy-6,4'-dihydroxy-3,5-di-(1,1-dimethylprop-2-enyl)chalcone
2-propoxy-6,4'-dihydroxy-3,5-dimethylchalcone
2-propoxy-6,4'-dihydroxy-3,5-diethylchalcone
2-propoxy-6,4'-dihydroxy-3,5-dipropylchalcone
2-propoxy-6,4'-dihydroxy-3,5-diisopropylchalcone
2-propoxy-6,4'-dihydroxy-3,5-di-t-butylchalcone
2-propoxy-6,4'-dihydroxy-3,5-di-(1,1-dimethylpropyl)chalcone
2-propoxy-6,4'-dihydroxy-3,5-diprop-2-enylchalcone
2-propoxy-6,4'-dihydroxy-3,5-dibut-2-enylchalcone
2-propoxy-6,4'-dihydroxy-3,5-di-(1,1-dimethylprop-2-enyl)chalcone
2-isopropoxy-6,4'-dihydroxy-3,5-dimethylchalcone
2-isopropoxy-6,4'-dihydroxy-3,5-diethylchalcone
2-isopropoxy-6,4'-dihydroxy-3,5-dipropylchalcone
2-isopropoxy-6,4'-dihydroxy-3,5-diisopropylchalcone
2-isopropoxy-6,4'-dihydroxy-3,5-di-t-butylchalcone
2-isopropoxy-6,4'-dihydroxy-3,5-di-(1,1-dimethylpropyl)chalcone
2-isopropoxy-6,4'-dihydroxy-3,5-diprop-2-enylchalcone
2-isopropoxy-6,4'-dihydroxy-3,5-dibut-2-enylchalcone
2-isopropoxy-6,4'-dihydroxy-3,5-di-(1,1-dimethylprop-2-enyl)chalcone
2-methoxy-6-hydroxy-4'-amino-3,5-dimethylchalcone
2-methoxy-6-hydroxy-4'-amino-3,5-diethylchalcone
2-methoxy-6-hydroxy-4'-amino-3,5-dipropylchalcone
2-methoxy-6-hydroxy-4'-amino-3,5-diisopropylchalcone
2-methoxy-6-hydroxy-4'-amino-3,5-di-t-butylchalcone
2-methoxy-6-hydroxy-4'-amino-3,5-di-(1,1-dimethylpropyl)chalcone
2-methoxy-6-hydroxy-4'-amino-3,5-diprop-2-enylchalcone
2-methoxy-6-hydroxy-4'-amino-3,5-dibut-2-enylchalcone
2-ethoxy-6-hydroxy-4'-amino-3,5-dimethylchalcone
2-ethoxy-6-hydroxy-4'-amino-3,5-diethylchalcone

2-ethoxy-6-hydroxy-4'-amino-3,5-dipropylchalcone
2-ethoxy-6-hydroxy-4'-amino-3,5-diisopropylchalcone
2-ethoxy-6-hydroxy-4'-amino-3,5-di-t-butylchalcone
2-ethoxy-6-hydroxy-4'-amino-3,5-di-(1,1-dimethylpropyl)chalcone
2-ethoxy-6-hydroxy-4'-amino-3,5-diprop-2-enylchalcone
2-ethoxy-6-hydroxy-4'-amino-3,5-dibut-2-enylchalcone
2-ethoxy-6-hydroxy-4'-amino-3,5-di-(1,1-dimethylprop-2-enyl)chalcone
2-propoxy-6-hydroxy-4'-amino-3,5-dimethylchalcone
2-propoxy-6-hydroxy-4'-amino-3,5-diethylchalcone
2-propoxy-6-hydroxy-4'-amino-3,5-dipropylchalcone
2-propoxy-6-hydroxy-4'-amino-3,5-diisopropylchalcone
2-propoxy-6-hydroxy-4'-amino-3,5-di-t-butylchalcone
2-propoxy-6-hydroxy-4'-amino-3,5-di-(1,1-dimethylpropyl)chalcone
2-propoxy-6-hydroxy-4'-amino-3,5-diprop-2-enylchalcone
2-propoxy-6-hydroxy-4'-amino-3,5-dibut-2-enylchalcone
2-propoxy-6-hydroxy-4'-amino-3,5-di-(1,1-dimethylprop-2-enyl)chalcone
2-isopropoxy-6-hydroxy-4'-amino-3,5-dimethylchalcone
2-isopropoxy-6-hydroxy-4'-amino-3,5-diethylchalcone
2-isopropoxy-6-hydroxy-4'-amino-3,5-dipropylchalcone
2-isopropoxy-6-hydroxy-4'-amino-3,5-diisopropylchalcone
2-isopropoxy-6-hydroxy-4'-amino-3,5-di-t-butylchalcone
2-isopropoxy-6-hydroxy-4'-amino-3,5-di-(1,1-dimethylpropyl)chalcone
2-isopropoxy-6-hydroxy-4'-amino-3,5-diprop-2-enylchalcone
2-isopropoxy-6-hydroxy-4'-amino-3,5-dibut-2-enylchalcone
2-isopropoxy-6-hydroxy-4'-amino-5-(1,1-dimethylprop-2-enyl)chalcone
2-methoxy-6-hydroxy-4'-methylamino-3,5-dimethylchalcone
2-methoxy-6-hydroxy-4'-methylamino-3,5-diethylchalcone
2-methoxy-6-hydroxy-4'-methylamino-3,5-dipropylchalcone
2-methoxy-6-hydroxy-4'-methylamino-3,5-diisopropylchalcone
2-methoxy-6-hydroxy-4'-methylamino-3,5-di-t-butylchalcone
2-methoxy-6-hydroxy-4'-methylamino-3,5-di-(1,1-dimethylpropyl)chalcone
2-methoxy-6-hydroxy-4'-methylamino-3,5-diprop-2-enylchalcone
2-methoxy-6-hydroxy-4'-methylamino-3,5-dibut-2-enylchalcone
2-ethoxy-6-hydroxy-4'-methylamino-3,5-dimethylchalcone
2-ethoxy-6-hydroxy-4'-methylamino-3,5-diethylchalcone
2-ethoxy-6-hydroxy-4'-methylamino-3,5-dipropylchalcone
2-ethoxy-6-hydroxy-4'-methylamino-3,5-diisopropylchalcone
2-ethoxy-6-hydroxy-4'-methylamino-3,5-di-t-butylchalcone
2-ethoxy-6-hydroxy-4'-methylamino-3,5-di-(1,1-dimethylpropyl)chalcone
2-ethoxy-6-hydroxy-4'-methylamino-3,5-diprop-2-enylchalcone
2-ethoxy-6-hydroxy-4'-methylamino-3,5-dibut-2-enylchalcone.

2-ethoxy-6-hydroxy-4'-methylamino-5-(1,1-dimethylprop-2-enyl)chalcone
2-propoxy-6-hydroxy-4'-methylamino-3,5-dimethylchalcone
2-propoxy-6-hydroxy-4'-methylamino-3,5-diethylchalcone
2-propoxy-6-hydroxy-4'-methylamino-3,5-dipropylchalcone
2-propoxy-6-hydroxy-4'-methylamino-3,5-diisopropylchalcone
2-propoxy-6-hydroxy-4'-methylamino-3,5-di-t-butylchalcone
2-propoxy-6-hydroxy-4'-methylamino-3,5-di-(1,1-dimethylpropyl)chalcone
2-propoxy-6-hydroxy-4'-methylamino-3,5-diprop-2-enylchalcone
2-propoxy-6-hydroxy-4'-methylamino-3,5-dibut-2-enylchalcone
2-propoxy-6-hydroxy-4'-methylamino-5-(1,1-dimethylprop-2-enyl)chalcone
2-isopropoxy-6-hydroxy-4'-methylamino-3,5-dimethylchalcone
2-isopropoxy-6-hydroxy-4'-methylamino-3,5-diethylchalcone
2-isopropoxy-6-hydroxy-4'-methylamino-3,5-dipropylchalcone
2-isopropoxy-6-hydroxy-4'-methylamino-3,5-diisopropylchalcone
2-isopropoxy-6-hydroxy-4'-methylamino-3,5-di-t-butylchalcone
2-isopropoxy-6-hydroxy-4'-methylamino-3,5-di-(1,1-dimethylpropyl)chalcone
2-isopropoxy-6-hydroxy-4'-methylamino-3,5-diprop-2-enylchalcone
2-isopropoxy-6-hydroxy-4'-methylamino-3,5-dibut-2-enylchalcone
2-isopropoxy-6-hydroxy-4'-methylamino-5-(1,1-dimethylprop-2-enyl)chalcone

and the corresponding ketones in which Z is selected from the groups (A)-(E) as defined above

2-methoxy-6,4'-dipivaloyloxy-3,5-dimethylchalcone
2-methoxy-6,4'-dipivaloyloxy-3,5-diethylchalcone
2-methoxy-6,4'-dipivaloyloxy-3,5-dipropylchalcone
2-methoxy-6,4'-dipivaloyloxy-3,5-diisopropylchalcone
2-methoxy-6,4'-dipivaloyloxy-3,5-di-t-butylchalcone
2-methoxy-6,4'-dipivaloyloxy-3,5-di-(1,1-dimethylpropyl)chalcone
2-methoxy-6,4'-dipivaloyloxy-3,5-diprop-2-enylchalcone
2-methoxy-6,4'-dipivaloyloxy-3,5-dibut-2-enylchalcone
2-ethoxy-6,4'-dipivaloyloxy-3,5-dimethylchalcone
2-ethoxy-6,4'-dipivaloyloxy-3,5-diethylchalcone
2-ethoxy-6,4'-dipivaloyloxy-3,5-dipropylchalcone
2-ethoxy-6,4'-dipivaloyloxy-3,5-diisopropylchalcone
2-ethoxy-6,4'-dipivaloyloxy-3,5-di-t-butylchalcone
2-ethoxy-6,4'-dipivaloyloxy-3,5-di-(1,1-dimethylpropyl)chalcone
2-ethoxy-6,4'-dipivaloyloxy-3,5-diprop-2-enylchalcone
2-ethoxy-6,4'-dipivaloyloxy-3,5-dibut-2-enylchalcone
2-ethoxy-6,4'-dipivaloyloxy-3,5-di-(1,1-dimethylprop-2-enyl)chalcone
2-propoxy-6,4'-dipivaloyloxy-3,5-dimethylchalcone

2-propoxy-6,4'-dipivaloyloxy-3,5-diethylchalcone
2-propoxy-6,4'-dipivaloyloxy-3,5-dipropylchalcone
2-propoxy-6,4'-dipivaloyloxy-3,5-diisopropylchalcone
2-propoxy-6,4'-dipivaloyloxy-3,5-di-t-butylchalcone
2-propoxy-6,4'-dipivaloyloxy-3,5-di-(1,1-dimethylpropyl)chalcone
2-propoxy-6,4'-dipivaloyloxy-3,5-diprop-2-enylchalcone
2-propoxy-6,4'-dipivaloyloxy-3,5-dibut-2-enylchalcone
2-propoxy-6,4'-dipivaloyloxy-3,5-di-(1,1-dimethylprop-2-enyl)chalcone
2-isopropoxy-6,4'-dipivaloyloxy-3,5-dimethylchalcone
2-isopropoxy-6,4'-dipivaloyloxy-3,5-diethylchalcone
2-isopropoxy-6,4'-dipivaloyloxy-3,5-dipropylchalcone
2-isopropoxy-6,4'-dipivaloyloxy-3,5-diisopropylchalcone
2-isopropoxy-6,4'-dipivaloyloxy-3,5-di-t-butylchalcone
2-isopropoxy-6,4'-dipivaloyloxy-3,5-di-(1,1-dimethylpropyl)chalcone
2-isopropoxy-6,4'-dipivaloyloxy-3,5-diprop-2-enylchalcone
2-isopropoxy-6,4'-dipivaloyloxy-3,5-dibut-2-enylchalcone
2-isopropoxy-6,4'-dipivaloyloxy-3,5-di-(1,1-dimethylprop-2-enyl)chalcone
2-methoxy-6,4'-dipivaloyloxymethoxy-3,5-dimethylchalcone
2-methoxy-6,4'-dipivaloyloxymethoxy-3,5-diethylchalcone
2-methoxy-6,4'-dipivaloyloxymethoxy-3,5-dipropylchalcone
2-methoxy-6,4'-dipivaloyloxymethoxy-3,5-diisopropylchalcone
2-methoxy-6,4'-dipivaloyloxymethoxy-3,5-di-t-butylchalcone
2-methoxy-6,4'-dipivaloyloxymethoxy-3,5-di-(1,1-dimethylpropyl)chalcone
2-methoxy-6,4'-dipivaloyloxymethoxy-3,5-diprop-2-enylchalcone
2-methoxy-6,4'-dipivaloyloxymethoxy-3,5-dibut-2-enylchalcone
2-ethoxy-6,4'-dipivaloyloxymethoxy-3,5-dimethylchalcone
2-ethoxy-6,4'-dipivaloyloxymethoxy-3,5-diethylchalcone
2-ethoxy-6,4'-dipivaloyloxymethoxy-3,5-dipropylchalcone
2-ethoxy-6,4'-dipivaloyloxymethoxy-3,5-diisopropylchalcone
2-ethoxy-6,4'-dipivaloyloxymethoxy-3,5-di-t-butylchalcone
2-ethoxy-6,4'-dipivaloyloxymethoxy-3,5-di-(1,1-dimethylpropyl)chalcone
2-ethoxy-6,4'-dipivaloyloxymethoxy-3,5-diprop-2-enylchalcone
2-ethoxy-6,4'-dipivaloyloxymethoxy-3,5-dibut-2-enylchalcone
2-ethoxy-6,4'-dipivaloyloxymethoxy-5-(1,1-dimethylprop-2-enyl)chalcone
2-propoxy-6,4'-dipivaloyloxymethoxy-3,5-dimethylchalcone
2-propoxy-6,4'-dipivaloyloxymethoxy-3,5-diethylchalcone
2-propoxy-6,4'-dipivaloyloxymethoxy-3,5-dipropylchalcone
2-propoxy-6,4'-dipivaloyloxymethoxy-3,5-diisopropylchalcone
2-propoxy-6,4'-dipivaloyloxymethoxy-3,5-di-t-butylchalcone
2-propoxy-6,4'-dipivaloyloxymethoxy-3,5-di-(1,1-dimethylpropyl)chalcone
2-propoxy-6,4'-dipivaloyloxymethoxy-3,5-diprop-2-enylchalcone

2-propoxy-6,4'-dipivaloyloxymethoxy-3,5-dibut-2-enylchalcone
2-propoxy-6,4'-dipivaloyloxymethoxy-5-(1,1-dimethylprop-2-enyl)chalcone
2-isopropoxy-6,4'-dipivaloyloxymethoxy-3,5-dimethylchalcone
2-isopropoxy-6,4'-dipivaloyloxymethoxy-3,5-diethylchalcone
2-isopropoxy-6,4'-dipivaloyloxymethoxy-3,5-dipropylchalcone
2-isopropoxy-6,4'-dipivaloyloxymethoxy-3,5-diisopropylchalcone
2-isopropoxy-6,4'-dipivaloyloxymethoxy-3,5-di-t-butylchalcone
2-isopropoxy-6,4'-dipivaloyloxymethoxy-3,5-di-(1,1-dimethylpropyl)chalcone
2-isopropoxy-6,4'-dipivaloyloxymethoxy-3,5-diprop-2-enylchalcone
2-isopropoxy-6,4'-dipivaloyloxymethoxy-3,5-dibut-2-enylchalcone
2-isopropoxy-6,4'-dipivaloyloxymethoxy-5-(1,1-dimethylprop-2-enyl)chalcone
2-methoxy-6,4'-(N,N-dimethylcarbamoyl)-3,5-dimethylchalcone
2-methoxy-6,4'-(N,N-dimethylcarbamoyl)-3,5-diethylchalcone
2-methoxy-6,4'-(N,N-dimethylcarbamoyl)-3,5-dipropylchalcone
2-methoxy-6,4'-(N,N-dimethylcarbamoyl)-3,5-diisopropylchalcone
2-methoxy-6,4'-(N,N-dimethylcarbamoyl)-3,5-di-t-butylchalcone
2-methoxy-6,4'-(N,N-dimethylcarbamoyl)-5-(1,1-dimethylpropyl)chalcone
2-methoxy-6,4'-(N,N-dimethylcarbamoyl)-3,5-diprop-2-enylchalcone
2-methoxy-6,4'-(N,N-dimethylcarbamoyl)-3,5-dibut-2-enylchalcone
2-ethoxy-6,4'-(N,N-dimethylcarbamoyl)-3,5-dimethylchalcone
2-ethoxy-6,4'-(N,N-dimethylcarbamoyl)-3,5-diethylchalcone
2-ethoxy-6,4'-(N,N-dimethylcarbamoyl)-3,5-dipropylchalcone
2-ethoxy-6,4'-(N,N-dimethylcarbamoyl)-3,5-diisopropylchalcone
2-ethoxy-6,4'-(N,N-dimethylcarbamoyl)-3,5-di-t-butylchalcone
2-ethoxy-6,4'-(N,N-dimethylcarbamoyl)-5-(1,1-dimethylpropyl)chalcone
2-ethoxy-6,4'-(N,N-dimethylcarbamoyl)-3,5-diprop-2-enylchalcone
2-ethoxy-6,4'-(N,N-dimethylcarbamoyl)-3,5-dibut-2-enylchalcone
2-ethoxy-6,4'-(N,N-dimethylcarbamoyl)-5-(1,1-dimethylprop-2-enyl)chalcone
2-propoxy-6,4'-(N,N-dimethylcarbamoyl)-3,5-dimethylchalcone
2-propoxy-6,4'-(N,N-dimethylcarbamoyl)-3,5-diethylchalcone
2-propoxy-6,4'-(N,N-dimethylcarbamoyl)-3,5-dipropylchalcone
2-propoxy-6,4'-(N,N-dimethylcarbamoyl)-3,5-diisopropylchalcone
2-propoxy-6,4'-(N,N-dimethylcarbamoyl)-3,5-di-t-butylchalcone
2-propoxy-6,4'-(N,N-dimethylcarbamoyl)-5-(1,1-dimethylpropyl)chalcone
2-propoxy-6,4'-(N,N-dimethylcarbamoyl)-3,5-diprop-2-enylchalcone
2-propoxy-6,4'-(N,N-dimethylcarbamoyl)-3,5-dibut-2-enylchalcone
2-propoxy-6,4'-(N,N-dimethylcarbamoyl)-5-(1,1-dimethylprop-2-enyl)chalcone
2-isopropoxy-6,4'-(N,N-dimethylcarbamoyl)-3,5-dimethylchalcone
2-isopropoxy-6,4'-(N,N-dimethylcarbamoyl)-3,5-diethylchalcone
2-isopropoxy-6,4'-(N,N-dimethylcarbamoyl)-3,5-dipropylchalcone
2-isopropoxy-6,4'-(N,N-dimethylcarbamoyl)-3,5-diisopropylchalcone

2-isopropoxy-6,4'-(N,N-dimethylcarbamoyl)-3,5-di-t-butylchalcone
2-isopropoxy-6,4'-(N,N-dimethylcarbamoyl)-5-(1,1-dimethylpropyl)chalcone
2-isopropoxy-6,4'-(N,N-dimethylcarbamoyl)-3,5-diprop-2-enylchalcone
2-isopropoxy-6,4'-(N,N-dimethylcarbamoyl)-3,5-dibut-2-enylchalcone
2-isopropoxy-6,4'-(N,N-dimethylcarbamoyl)-5-(1,1-dimethylprop-2-enyl)chalcone

Specific examples of bis-aromatic α,β-unsaturated ketones are

2-methoxy-4,4'-dihydroxy-5-propylchalcone
2-methoxy-4,4'-dihydroxy-5-(1,1-dimethylpropyl)chalcone
2-methoxy-4,4'-dihydroxy-5-prop-2-enylchalcone
2-methoxy-4,4'-dihydroxy-5-but-2-enylchalcone

2-ethoxy-4,4'-dihydroxy-5-propylchalcone
2-ethoxy-4,4'-dihydroxy-5-(1,1-dimethylpropyl)chalcone
2-ethoxy-4,4'-dihydroxy-5-prop-2-enylchalcone
2-ethoxy-4,4'-dihydroxy-5-but-2-enylchalcone
2-ethoxy-4,4'-dihydroxy-5-(1,1-dimethylprop-2-enyl)chalcone

2-propoxy-4,4'-dihydroxy-5-propylchalcone
2-propoxy-4,4'-dihydroxy-5-(1,1-dimethylpropyl)chalcone
2-propoxy-4,4'-dihydroxy-5-prop-2-enylchalcone
2-propoxy-4,4'-dihydroxy-5-but-2-enylchalcone
2-propoxy-4,4'-dihydroxy-5-(1,1-dimethylprop-2-enyl)chalcone

2-isopropoxy-4,4'-dihydroxy-5-propylchalcone
2-isopropoxy-4,4'-dihydroxy-5-(1,1-dimethylpropyl)chalcone
2-isopropoxy-4,4'-dihydroxy-5-prop-2-enylchalcone
2-isopropoxy-4,4'-dihydroxy-5-but-2-enylchalcone
2-isopropoxy-4,4'-dihydroxy-5-(1,1-dimethylprop-2-enyl)chalcone

2-methoxy-4-hydroxy-4'-amino-5-propylchalcone
2-methoxy-4-hydroxy-4'-amino-5-(1,1-dimethylpropyl)chalcone
2-methoxy-4-hydroxy-4'-amino-5-prop-2-enylchalcone
2-methoxy-4-hydroxy-4'-amino-5-but-2-enylchalcone

2-ethoxy-4-hydroxy-4'-amino-5-propylchalcone
2-ethoxy-4-hydroxy-4'-amino-5-(1,1-dimethylpropyl)chalcone
2-ethoxy-4-hydroxy-4'-amino-5-prop-2-enylchalcone
2-ethoxy-4-hydroxy-4'-amino-5-but-2-enylchalcone
2-ethoxy-4-hydroxy-4'-amino-5-(1,1-dimethylprop-2-enyl)chalcone

prepared by reacting the corresponding phenols (e.g. IIa, IIb, IIIa) with an activated ester (including the α-halomethylesters), an anhydride or, preferably, an acid halogenide, in particular the acid chloride.

The reaction is performed in an aprotic organic solvent such as lower aliphatic ketones like acetone, butanone, aliphatic ethers like tetrahydrofuran, diethylether, or dioxane or a liquid amine like pyridine.

The reaction is carried out in the presence of an acid scavenger such as potassium or sodium carbonate, an tertiary aliphatic amine such as triethylamine, or pyridine.

An especially spectacular modification of the method involves the reaction of the phenol with the appropriate anhydride using 4-dimethylaminopyridine or 4-(1-pyrrolidino)pyridine as catalyst. With these reaction conditions, the reaction gives a very high yield.

N,N-Dimethylcarbamic esters of the phenols of the general formula I (I'Bb, IIIBa) may be prepared by reacting the corresponding phenols of the general formula I (IIa, IIb) with an activated derivative of N,N-dimethylcarbamic acid such as an activated ester or, preferably, an acid halide, in particular the acid chloride.

The reaction is carried out in an aprotic organic solvent such as lower aliphatic ketones like acetone, butanone, aliphatic ethers such as tetrahydrofuran, diethylether, or dioxane, or a liquid amine such as pyridine, or a liquid nitrile such as acetonitrile.

In general, the reaction is carried out in the presence of an acid scavenger such as potassium or sodium carbonate, a tertiary aliphatic amine such as triethylamine or pyridine

Alternatively, the N,N-dimethylcarbamoyl esters may be prepared by condensing the carbamoylated phenolic benzaldehydes or phenolic acetophenones with the appropriate acetophenones or benzaldehydes, respectively.

The alkoxymethoxy ethers of the general formula I (I'Cb, IICa, IICb, IIICa) are most conveniently prepared by condensing the appropriate ethers of the phenolic benzaldehydes or the phenolic acetophenones with the appropriate acetophenones or benzaldehydes, respectively.

They may, however, be prepared by reacting the phenolic chalcones with the appropriate alkyl-α-alkylhalomethyl halide.

The reaction may be carried out in an aprotic organic solvent like a lower aliphatic ketone, such as acetone or butanone, or an ether, such as tetrahydrofuran, dioxane or dioxolane or a liquid nitrile such as acetonitrile.

The reaction may be performed in the presence of a acid scavenger such as an inorganic or organic base. The base may be potassium or sodium or quaternary ammonium carbonate, or hydroxide.

LEGENDS TO THE FIGURES

Fig. 1 shows the effect of licochalcone A on intracellular survival of *Leishmania major* vaccine strain in U937 cells measured by the parasite survival index (PSI).

Fig. 2 shows the effect of licochalcone A on intracellular survival of *Leishmania major* vaccine strain in human peripheral blood monocytes derived macrophages measured by the parasite survival index (PSI).

Fig. 3 shows the effect of licochalcone A on the parasitic load of the footpad of the mice infected with *Leishmania major* as described in example 8.

Fig. 4A and 4B show the effect of licochalcone A on the *in vitro* growth of chloroquine-resistant *Plasmodium falciparum* and chloroquine-sensitive *Plasmodium falciparum*, respectively as described in example 14.

Fig. 5 is an electron microscopic photo (magnification 10,000x) showing *Leishmania major* promastigotes as a control. *Leishmania major* promastigotes are shown with normal mitochondria. Mitochondria denoted "M".

Fig. 6 is an electron microscopic photo (magnification 10,000x9 showing *Leishmania major* promastigotes after incubation with 10 µg/ml of licochalcone A. From the photo it is seen that the mitochondria are swollen to an extent that made it difficult to recognize the structures as mitochondria, if not the characteristic cristae had been preserved. Mitochondria denoted "M".

Fig. 7 is a flow sheet illustrating the isolation of various cell types from peripheral blood.

Fig. 8. Five Syrian golden male hamsters weighing 50-70 g which were infected with *L. donovani* by intracardial injection of 2 x 10^7 stationary phase promastigotes. One day later the animals were injected intraperitoneally with 10 mg/kg body weight licochalcone A (100 µl in saline) for 6 days. The animals were sacrificed on day 8 and

parasite load in the spleen and liver was determined by determining the growth of promastigotes from the spleen and the liver using ^{3}H-thymidine uptake by promastigotes.

Fig. 9A-C show the effect of licochalcone A on the *in vitro* growth of *L. major* WHO pentostam resistant strain promastigotes. 3 x10^6 promastigotes were incubated in the presence of licochalcone A (µg/ml, Fig. 9A), pentostam (x 100 µg/ml, Fig. 9B) and licochalcone A (µg/ml) plus pentostam (10 µg/ml) (Fig. 9C) for 2 h followed by 18 h uptake of ^{3}H-thymidine. The results are based on 5 experiments and are given as growth index (mean±SEM) as measured by ^{3}H-thymidine uptake by promastigotes.

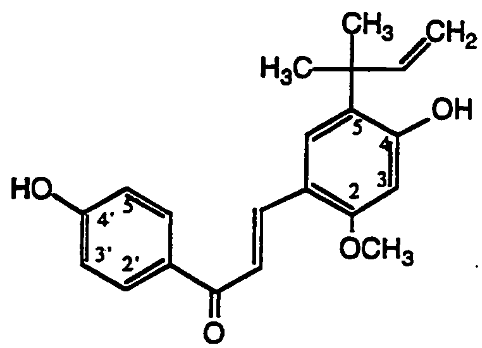
Fig. 10A-B show the effect of licochalcone A on the *in vitro* growth of *L. major* promastigotes from 4-days cultures. The results are based on 5 experiments and are given as growth index (mean±SEM, Fig. 10A) as measured by ^{3}H-thymidine uptake and flagellar motility (mean±SEM, Fig. 10B) of parasites as determined microscopically by counting 500 promastigotes.

Fig. 11A-C show an electron micrograph of a human macrophage showing a macrophage incubated in the presence of 10 µg /ml licochalcone A for 24 h. The figure shows several mitochondria (small arrows, A) and a higher magnification of one of the mitochondria (B). The ultrastructure of this mitochondrion shows longitudinally arranged cristae (small arrowheads) and general appearance similar to the mitochondrion from a macrophage grown in the presence of medium alone (C).

EXAMPLE 1

Isolation of bis-aromatic α,β-unsaturated ketone from Chinese licorice root of Glycyrrhiza species rich in licochalcone A (a batch of *G. uralensis* or *G. inflata*) by bioassay-guided fractionation

1) Isolation of licochalcone A



The test for parasitic activity referred to in this example was performed as the *in vitro L. major* growth test described in Example 4.

Comminuted dried roots of chinese licorice roots rich in licochalcone A (a batch of *G. uralensis* or *G. inflata*) (674 g) were extracted with ethanol (2 l) for 24 hours. The mixture was filtered and the filtrate concentrated *in vacuo* to give 64 g of a gum. The gum was partitioned between 0.5 l of water and 0.5 l of methylene chloride-methanol (1:1), and the two phases concentrated *in vacuo*. Only the residue from the organic phase showed a major activity against the parasite.

The residue from the organic phase was concentrated *in vacuo* and partitioned between methanol-water (9:1) (150 ml) and hexane (150 ml) and the two phases concentrated *in vacuo*. Only the residue from the methanolic phase showed a major activity against the parasite.

The residue from the methanolic phase was partitioned between methanol-water (3:2) (400 ml) and methylene chloride (400 ml) and the two phases concentrated *in vacuo*. The residues from both phases (a total of 27 g) showed activity against the parasite and were combined.

A sample of the residue from the methylene chloride phase (4 g) was chromatographed over silica gel 60 (Merck 0.063-0.200 mm, 400 g) using toluene-ethyl acetate

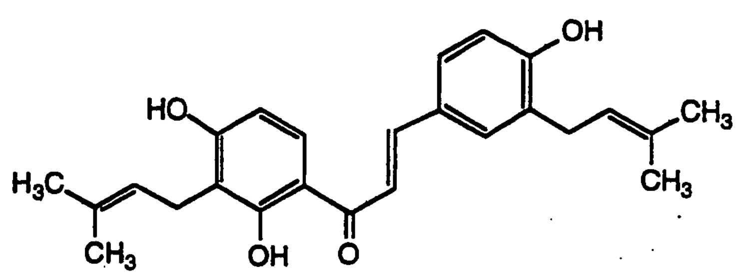
(9:1, 300 ml), (4:1, 100 ml), (3:2, 100 ml), (1:4, 100 ml), and ethyl acetate to which increasing amounts of methanol were added as eluents.

The residue (1.5 g after concentration *in vacuo*) of the fractions which eluted while pure ethyl acetate was added as eluent showed the major activity. The residue was chromatographed over silica gel (150 g) using methylene chloride-ethyl acetate (14:1, 100 ml), (9:1, 100 ml), (4:1, 100 ml), (3:2, 100 ml), and (2:3, 100 ml) as eluents.

The residue (0.6 g of a gum after concentration *in vacuo*) of the fractions which eluted while methylene chloride-ethyl acetate (4:1) was added showed the major activity against the parasite. Licochalcone A (0.3 g) was obtained by crystallization of the residue from methanol-water, m.p. 101-102°C (T. Saitoh, and S. Shibata in *Tetrahedron Lett.* 50 (1975), 4461: m.p. 101-102°C).

Recrystallisation afforded a product, m.p. 136-138°C (polymorphic) (X. Rhen-Seng, W. Kung-Ling, J. Shifa, W. Chang-gen, J. Fu-Xiang, X. Xu-yan, and G. Yi-Sheng, in *Acta Chem. Sinica* 37 (1979), 289-297 ref. X. R. Sheng: m.p. 136-138°C). The ^{1}H NMR data agreed with those published for licochalcone A by T. Saitoh, and S. Shibata.

2) Isolation of 4,2',4'-trihydroxy-3,3'-di-(3-methylbut-2-enyl)chalcone



Comminuted dried roots of a sample of chinese licorice roots (1 kg) possessing only a low amount of licochalcone A (*G. uralensis*) were extracted with ethanol (5 l) for 19 h. The mixture was filtered and the filtrate concentrated *in vacuo* to give 76.5 g of a gum. The gum was partitioned between 500 ml of water and 250 ml of ether. The aqueous phase was extracted with an additional 450 ml of ether and the combined organic phases concentrated *in vacuo* to give 54 g of a gum. Only the residue from the organic phase showed a major activity against the parasite.

The residue from the organic phase was chromatographed over silica gel 60 (Merck 0.063-0.200 mm, 800 g) using toluene-ethyl acetate (9:1, 0.25 l), (8:2, 1 l), (7:3, 1 l), (6:4, 1

l), (1:1, 1 l), and finally pure ethyl acetate (1 l) as eluents.

The residue (1.82 g of a yellow gum after concentration *in vacuo*) of the fractions which eluted while toluene-ethyl acetate (8:2) was added showed the major activity. The gum was chromatographed over silica gel 60 (Merck 0.063-0.200 mm, 200 g) using methylene chloride (1.5 l), methylene chloride-ethyl acetate (9:1, 1 l) and (8:2, 0.5 l) as eluents.

The residue (0.25 g of a yellow gum after concentration *in vacuo*) of the fractions which eluted while methylene chloride-ethyl acetate (9:1) was added showed the major activity. The gum was chromatographed over silica gel 60 (Merck 0.063-0.200 mm, 25 g) using petroleum ether-ethyl acetate (8:2, 250 ml), (7:3, 200 ml), and (1:1, 100 ml) as eluents.

The residue (42.4 mg of a yellow gum after concentration *in vacuo*) of the fractions which eluted while petroleum ether-ethyl acetate (1:1) was added showed the major activity. The gum was chromatographed by high performance liquid chromatography over LiChrosorp RP 18 (Knauer 16 X 250 mm, 10 µm) using acetonitrile-water (8:2, flow rate 9.9 ml/min) as an eluent. Two amorphous yellow compounds were obtained, in which no impurities could be observed by ^1H NMR spectroscopy (200 MHz). The first compound was by ^1H and ^{13}C NMR, by UV spectroscopy, and by mass spectroscopy shown to be the not previously characterized (E)-1-(2,4-dihydroxy-3-(3-methyl-2-butenyl)phenyl-3-(2,2-dimethyl-8-hydroxy-2H-benzopyran-1-yl)-2-propen-1-one (A) and the second was by ^1H and ^{13}C NMR, by UV spectroscopy, and by mass spectroscopy shown to be identical with the 4,2',4'-trihydroxy-3,3'-di-(3-methylbut-2-enyl)chalcone described by K. Kyogoku, K. Matayama, S. Yokomori, R. Saziki, S. Nakame, N. Sasajima, J Sawada, M. Ohzeki and I. Tanaka in *Chem. Pharm. Bull.* 27 (1979), 2943. Only the latter compound showed major antiparasitic effects.

EXAMPLE 2

Synthesis of intermediates for use in the preparation of bis-aromatic α,β-unsaturated ketones

1) <u>Preparation of 4-((3-methyl)but-2-enyloxy)-2-hydroxybenzaldehyde</u>

A solution of 6 g (0.11 mol) of potassium hydroxide, 22.7 g (0.1 mol) of benzyltriethylammonium chloride, and 14 g (0.1 mol) of 2,4-dihydroxybenzaldehyde was concentrated *in vacuo*. The residue was suspended in 100 ml of ethyl acetate and 11.7 ml (0.1 mol) of dimethylallyl bromide was slowly added to the solution. The mixture was extracted with 100 ml of water, and the organic phase was dried and concentrated *in*

vacuo to give 3.568 g of a residue, from which 4.738 g (20%) of 4-(3-methyl)-but-2-enyl-oxy-2-hydroxybenzaldehyde identical to that previously described by S. Khan, and M. Krishnamurti in *Indian J. Chem.* **22B** (1983), 276 was purified by column chromatography over silica gel 60 (Merck 0.063-0.200 mm, 200 g) using petroleum ether-ethyl acetate (9:1) as an eluent.

2) Preparation of 4-((3-methyl)but-2-enyloxy)-2-methoxybenzaldehyde

4 g (2 mmol) of 4-((3-methyl)but-2-enyloxy)-2-hydroxybenzaldehyde was added to a suspension of 11.4 g of potassium carbonate in 46 ml of acetone and the suspension was refluxed for 6 h. The suspension was filtered and the filtrate concentrated to give 4.701 g of an oil, from which 2.53 g (60%) of 4-((3-methyl)but-2-enyloxy)-2-methoxybenzaldehyde identical to that previously described by S. A. Khan, and M. Krishnamurti in *Indian J. Chem.* **22B** (1983), 276-277 was isolated by column chromatography over silica gel 60 (Merck 0.063-0.200 mm, 100 g) using petroleum ether-ethyl acetate (9:1) as an eluent.

3) Preparation of 4-(N,N-dimethylcarbamoyloxy)acetophenone

1.35 ml (15 mmol) of dimethylcarbamoyl chloride and 1.36 g (10 mmol) 4-hydroxyacetophenone were added to a suspension of 5 g of potassium carbonate in 50 ml of acetone. The mixture was left for 2 h under stirring and filtered, and the filtrate was concentrated *in vacuo* to give a residue, from which 1.13 g (60%) of 4-(N,N-dimethylcarbamoyloxy)acetophenone was obtained by crystallization from methanol.

^{1}H NMR (200 MHz, CD_3CN, δ) 7.98 (AA'-part of an AA'MM'-system, H-2 and H-6), 7.21 (MM'-part of an AA'MM'-system, H-3 and H-5), 3.07 and 2.92 (CH_3-N), 2.56 (CH_3-C).

4) Preparation of 3-(2,4-dimethoxyphenyl)-1-(4-hydroxyphenyl)propan-1-on

A solution of 568 mg (2 mmol) of 2,4-dimethoxy-4'-hydroxychalcone in ethanol (20 ml) was added 50 mg of platinum on charcoal (10%) and hydrogenated at 20 atm for 18 h. The colourless solution was filtered and concentrated *in vacuo* to give 606 mg, from which 143.7 mg (24%) of 3-(2,4-dimethoxyphenyl)-1-(4-hydroxyphenyl)propan-1-on was isolated by column chromatography over silica gel 60 (Merck 0.063-0.200 mm, 60 g) using petroleum ether-ethyl acetate (4:1, 500 ml, 3:1, 500 ml) as eluents, m.p. 126.4-127.4°C.

^{1}H NMR data (200 MHz, $CDCl_3$, δ) 7.91 (AA'-part of an AA'MM'-system, H-2' and H-6'), 7.07 (d, *J* 8 Hz, H-6), 6.88 (MM'-part of an AA'MM'-system, H-3' and H-5'), 6.43 (d, *J*

3 Hz, H-3), 6.40 (dd, *J* 8 and 3 Hz, H-5) 3.83 and 3.78 (s, CH_3O), 3.17 (perturbed t, H-2), 2.96 (perturbed t, H-3).

Calc. for $C_{17}H_{18}O_4$: C 71.31, H 6.34. Found: C 71.55, H 6.35.

5) Preparation of 2,4-diprop-2-enyloxybenzaldehyde

To a solution of 6.9 g (50 mmol) of 2,4-dihydroxybenzaldehyde in 100 ml of acetone was added 12 ml (120 mmol) of 3-bromopropene and 40 g of potassium carbonate and the mixture was refluxed for 3 h under stirring. The mixture was filtered and the filtrate concentrated *in vacuo* to give 10.2 g (93%) of 2,4-diprop-2-enyloxybenzaldehyde as slightly reddish crystals.

^1H NMR data (200 MHz, $CDCl_3$, δ) 10.35 (s, CHO), 7.80 (d, *J* 10 Hz, H-6), 6.57 (dd, *J* 3 and 10 Hz, H-5), 6.47 (d, *J* 3 Hz, H-3), 6.2-5.9 (m, 2 CH= groups), 5.6-5.2 (2 CH_2= groups), 4.63 (2 CH_2O groups).

^{13}C NMR data (50 MHz, δ, $CDCl_3$) 187.5, 12.9, 159.2, 156.5, 142.5, 130.7.

6) Preparation of 2,4-dihydroxy-5-alkylbenzaldehydes or 2,4-dihydroxy-3,5-dialkylbenzaldehydes

Many of these compounds are already known and can generally be prepared by reacting the corresponding 1,3-dihydroxy-4-alkylbenzene or the corresponding 1,3-dihydroxy-3,5-dialkylbenzaldehyde with hydrogen cyanide or zinc cyanide in an ether solution in the presence of hydrogen chloride followed by hydrolysis of the formed product. Alternatively, the products may be formed through a Vilsmeier-Haack reaction (see J. March, "Advanced Organic Chemistry", 4th Ed., John Wiley & Sons, New York, 1992, 542-543).

7) Preparation of 2-hydroxy-4-alk-2-enyloxy-5-alkylbenzaldehydes or 2-hydroxy-3,5-dialkyl-4-alk-2-enyloxybenzaldehydes

The appropriate 3,5-dialkyl-2,4-dihydroxybenzaldehyde or 5-alkyl-2,4-dihydroxybenzaldehyde is selectively alkylated at the 4-hydroxy group with an alk-2-enyl bromide according to the procedure described for the synthesis of 4-((3-methyl)but-2-enyloxy)-2-hydroxybenzaldehyde (see Example 2.1).

8) Preparation of 2-methoxy-4-alk-2-enyloxy-5-alkylbenzaldehydes or 2-methoxy-3,5-dialkyl-4-alk-2-enyloxybenzaldehydes

The appropriate 4-alk-2-enyloxy-2-hydroxybenzaldehyde is alkylated with dimethyl sulfate according to the procedure described for the synthesis of 4-((3-methyl)but-2-enyloxy)-2-hydroxybenzaldehyde (see Example 2.1).

9) Preparation of 2-methoxy-5-alkyl-4-hydroxybenzaldehydes or 2-methoxy-3,5-dialkyl-4-hydroxybenzaldehydes

An acidic methanolic solution of the appropriate 2-methoxy-4-alk-2-enyloxy-5-alkyl-benzaldehyde or 2-methoxy-3,5-dialkyl-4-alk-2-enyloxybenzaldehyde to which has been added a small amount of water is heated in the presence of palladium on carbon to give the title compound as described in Example 2.22.

10) Preparation of 3,5-dialkyl-2,6-dihydroxybenzaldehydes

A general procedure for the preparation of 3,5-dialkyl-2,6-dihydroxybenzaldehyde comprises reacting the appropriate 1,3-dihydroxy-4,6-dialkylbenzene with hydrogen cyanide or zinc cyanide in an ether solution in the presence of hydrogen chloride followed by hydrolysis of the resulting product. Alternatively, the products may be prepared through a Vilsmeier-Haack reaction (see J. March, "Advanced Organic Chemistry", 4th Ed., John Wiley & Sons, New York, 1992, 542-543).

11) Preparation of 3,5-dialkyl-2-methoxy-6-hydroxybenzaldehydes

An acetone solution of the appropriate 3,5-dialkyl-2,6-dihydroxybenzaldehyde is treated with an equimolar amount of methyl iodide or dimethyl sulfate in the presence of potassium carbonate (cf. the synthesis described in Example 2.1).

12) Preparation of 2-methoxy-4-hydroxy-5-alk-2-enylbenzaldehydes

A Claisen rearrangement of the appropriate 2-methoxy-4-alk-2-enyloxybenzaldehyde is performed as described for the synthesis of 2-methoxy-4-hydroxy-5-(1,1-dimethylprop-2-enyl)benzaldehyde (see Example 2.16).

13) Preparation of 2-alk-2-enyloxy-6-hydroxybenzaldehydes

An acetone solution of 2,6-dihydroxybenzaldehyde is treated with an equimolar amount of an alky-2-enyl bromide in the presence of potassium carbonate to give the title compound (cf. Example 2.1).

14) Preparation of 2-alk-2-enyloxy-6-methoxybenzaldehydes

An acetone solution of the appropriate 2-alk-2-enyloxy-6-hydroxybenzaldehyde is treated with dimethyl sulfate in the presence of potassium carbonate as described in the synthesis of 2-methoxy-4-(3-methylbut-2-enyloxy)benzaldehyde (Example 2.16).

15) Preparation of 2-methoxy-5-alk-2-enyl-6-hydroxybenzaldehydes

The appropriate 2-alk-2-enyloxy-6-methoxybenzaldehyde is Claisen rearranged as described for the synthesis of 2-methoxy-4-hydroxy-5-(1,1-dimethylprop-2-enyl)benzaldehyde (Example 2.16).

Synthesis of bis-aromatic α,β-unsaturated ketones

16) Synthesis of licochalcone A

Two methods for the preparation of licochalcone A are described, one by S. A. Khan, and M. Krishnamurti in *Indian J. Chem.* 22B (1983), 276-277, and one by X. Ren-Sheng, W. Kung-Ling, J. Shifa, W. Chang-gen, J. Fu-Xiang, X. Xu-yan, and G. Yi-Sheng in *Acta Chem. Sinica* 37 (1979), 289-297. The key steps in the Indian method are a Claisen condensation between 4-methoxymethoxybenzaldehyde and 2-methoxy-4-(3-methylbut-2-enyloxy)chalcone followed by a Claisen rearrangement to give 4'-O-methoxymethyllicochalcone A. In the Chinese approach the key step is an acid catalyzed Claisen condensation of 4-hydroxybenzaldehyde with 2-methoxy-4-hydroxy-5-(1,1-dimethylprop-2-enyl)benzaldehyde to give licochalcone A. The possibility for a contamination of the methoxy-methyl chloride, used for preparation of methoxymethyl ethers with the highly carcinogenic dichloromethyl ether, made the Chinese method preferable. The starting material, 2-hydroxy-4-hydroxy-5-(1,1-dimethylprop-2-enyl)benzaldehyde, was prepared as described below:

34.5 g (250 mmol) of 2,4-dihydroxybenzaldehyde and 14 g (250 mmol) of potassium hydroxide were dissolved in 500 ml of ethanol. To the solution was added 57 g (250 mmol) of benzyltriethylammonium chloride, and the solution was concentrated *in vacuo*. The residue was dissolved in 500 ml of ethyl acetate and to the solution was over 30 min added a solution of 3-methylbut-2-enyl bromide dissolved in 100 ml of ethyl acetate. The mixture was refluxed for 1 hour and filtered, and the filtrate admixed with 500 ml of ether. The mixture was extracted with 500 ml of water, the organic phase was concentrated *in vacuo* to give 46 g of an yellow oil, from which 10.6 g of 2-hydroxy-4-(3-methylbut-2-enyloxy)benzaldehyde was isolated by column chromatography over silica gel 60 (Merck 0.063-0.200 mm, 1000 g) using petroleum ether-ethyl acetate (14:1, 1200 ml), (9:1, 1000 ml), (7:1, 1000 ml), (5:1, 300 ml) as eluents.

2-Methoxy-4-(3-methylbut-2-enyloxy)benzaldehyde was prepared as described below:

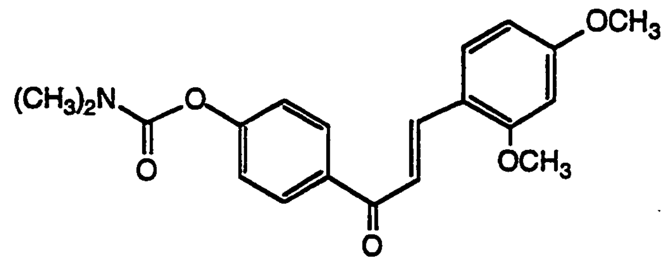
10.6 g (206 mmol) of 2-hydroxy-4-(3-methylbut-2-enyloxy)benzaldehyde and 5.4 ml of dimethyl sulfate were dissolved in 102 ml of distilled acetone, the solution was added 25.5 g of potassium carbonate, and the resulting mixture was refluxed for 5 hours. The mixture was filtered and the filtrate concentrated *in vacuo* to give 8.3 g of an yellow oil from which 5.4 g of 2-methoxy-4-(3-methylbut-2-enyloxy)benzaldehyde was isolated by column chromatography over silica gel 60 (Merck 0.063-0.200 mm, 400 g) using petroleum ether-ethyl acetate (9:1, 1000 ml), (4:1, 500 ml) as eluents.

The Claisen rearrangement of 2-methoxy-4-(3-methylbut-2-enyloxy)benzaldehyde and the Claisen condensation of the resulting 2-methoxy-4-hydroxy-5-(1,1-dimethyl-prop-2-enyl)benzaldehyde with 4-hydroxybenzaldehyde to give licochalcone A was performed according to the Chinese procedure:

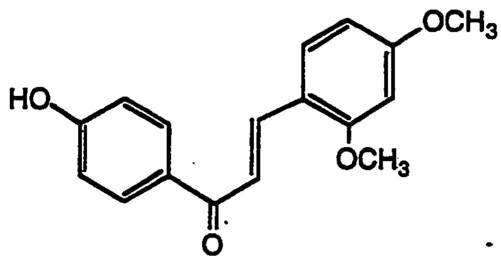
0.8 g (3.6 mmol) of 2-methoxy-4-(3-methylbut-2-enyl)benzaldehyde and 8.8 ml of propionic anhydride were dissolved in 17 ml of freshly distilled N,N-dimethylaniline and the solution was left under argon atmosphere in a sealed glass vessel for 2.5 hours at 200°C. 20 ml was removed from the reaction mixture by Kugelrohr distillation (oven 100°C, 1 mmHg), and to the residue was added 15 ml of water and sulfuric acid until pH 4. The mixture was extracted with two 40 ml portions of ether and the extract was concentrated *in vacuo* to give 1.1 g of an oil from which 0.398 g of 2-methoxy-4-hydroxy-5-(1,1-dimethylprop-2-enyl)benzaldehyde was isolated by column chromatography over silica gel 60 (Merck 0.063-0.200 mm, 80 g) using petroleum ether-ethyl acetate (9:1, 1000 ml) as an eluent.

A solution of 150 mg (1.1 mmol) of 4-hydroxyacetophenone and 220 mg (1.0 mmol) of 2-methoxy-4-hydroxy-5-(1,1-dimethylprop-2-enyl)benzaldehyde in 3 ml of ethanol was left on an ice bath, and 1.1 ml of ethanol saturated with dry hydrogen chloride was added. The solution was left for 2 hours and poured into 10 ml of water. The mixture was concentrated *in vacuo* and extracted with two 10 ml portions of ethyl acetate, and the extract was dried and concentrated *in vacuo* to give 281 mg of an oil from which 119 mg of licochalcone A was isolated by column chromatography over silica gel 60 (Merck 0.063-0.200 mm, 23 g) using petroleum ether-ethyl acetate (1:1, 1000 ml) as an eluent.

17) Preparation of 2,4-dimethoxy-4'-(N,N-dimethylcarbamoyloxy)chalcone



and 2,4-dimethoxy-4'-hydroxychalcone

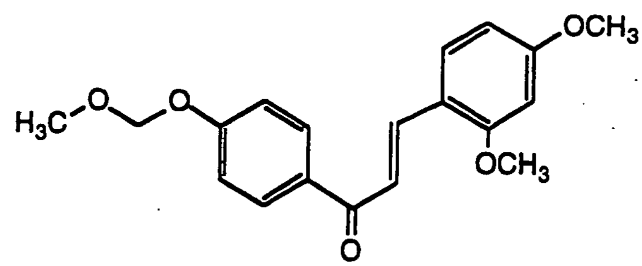


414 mg (2 mmol) 4-N,N-dimethylcarbamoyloxyacetophenone and 166 mg (1 mmol) 2,4-dimethoxybenzaldehyde were added to a solution of 1 g of potassium hydroxide in ethanol and the mixture was left for 1 h. The mixture was filtered and the residue poured into 20 ml of 2 M hydrochloric acid. The solution was extracted twice with 20 ml of ethyl acetate. The organic phase was dried and concentrated *in vacuo* to give 0.906 g of an yellow oil, from which 0.157 g of 2,4-dimethoxy-4'-hydroxychalcone and 47 mg of 2,4-dimethoxy-4'(N,N-dimethylcarbamoyloxy)chalcone were isolated by column chromatography over silica gel 60 (Merck 0.063-0.200 mm, 50 g) using petroleum ether-ethyl acetate (2:1) as an eluent.

^{1}H NMR data for 2,4-dimethoxy-4'-(N,N-dimethylcarbamoyloxy)chalcone (200 MHz, δ, CD_3CN): 8.06 (AA'-part of an AA'MM'-system, H-2' and H-6'), 8.00 (d, *J* 15 Hz, H-β), 7.71 (d, *J* 7 Hz, H-6), 7.63 (d, *J* 15 Hz, H-α), 7.24 (MM'-part of an AA'MM'-system, H-3' and H-5'), 6.59 (dd, *J* 7 and 2 Hz, H-5), 6.57 (d, *J* 2 Hz, H-3), 3.91 and 3.94 (s, CH_3-O), 3.05 and 2.95 (s, CH_3-N).

For the identification of 2,2-dimethoxy-4'-hydroxy, see Example 2.22.

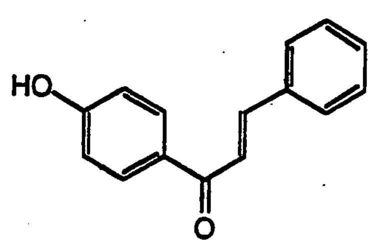
18) Preparation of 2,4-dimethoxy-4'-methoxymethoxychalcone



0.9 g (5 mmol) of 4-methoxymethoxyacetophenone and 1.66 g (10 mmol) of 2,4-dimethoxybenzaldehyde were added to a solution of 5 g of potassium hydroxide in ethanol and the solution was left for 2.5 h. The reaction mixture was poured into a mixture of 20 ml of a saturated sodium bicarbonate solution and 20 ml of water. The mixture was extracted twice with ether and the combined organic phases were dried and concentrated *in vacuo* to give 4.621 g of an oil from which 1.60 g (90%) of 2,4-dimethoxy-4'-methoxymethoxychalcone was isolated by column chromatography over silica gel 60 (Merck 0.063-0.200 mm, 100 g) using petroleum ether-ethyl acetate (4:1, 1 l) and (2:1, 1 l) as eluents.

^{1}H NMR data (200 MHz CD_3CN, δ,): 8.03 (AA'-part of an AA'MM'-system, H-2' and H-6'), 7.96 (d, *J* 15 Hz, H-β), 7.70 (d, *J* 9.3 Hz, H-6), 7.63 (d, *J* 15 Hz, H-α), 7.11 (MM'-part of an AA'MM'-system, H3'and H-5'), 6.58 (dd, *J* 9.3 and 2.6 Hz, H-5), 6.56 (d, *J* 2.6 Hz, H-3), 5.26 (s, CH_2), 3.90 and 3.83 (s, CH_3O-Ph), 3.44 (CH_3-O-CH_2).

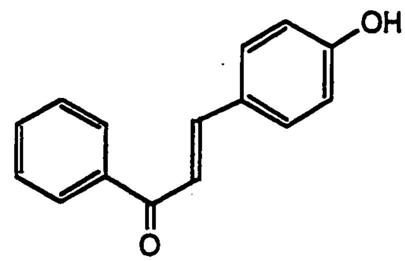
19) Preparation of 4'-hydroxychalcone



2.7 g (20 mmol) of 4-hydroxyacetophenone and 2.1 g (20 mmol) of benzaldehyde were added to a solution of 12 g of sodium hydroxide in 10 ml of water and 50 ml of ethanol, and the solution was refluxed for 3 h. After addition of 50 ml of water, the solution was acidified with 90 ml of 4 M hydrochloric acid and extracted with 100 ml of ether. The organic phase was dried ($MgSO_4$) and concentrated *in vacuo* to give 4.82 g of a gum, from which 0.124 g of 4'-hydroxychalcone identical with that previously de-

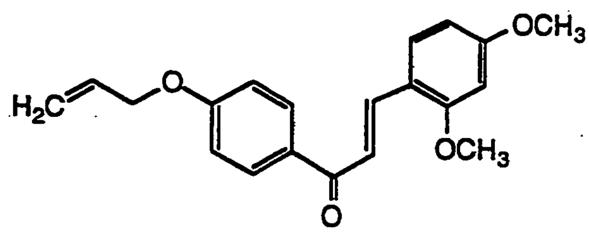
scribed by R. L. Shriner and T. Kurosawa in *J. Am. Chem. Soc.* 52 (1930), 2538-2540 was obtained by column chromatography over silica gel 60 (Merck 0.063-0.200, 300 g) using methylene chloride-ethyl acetate (9:1) as an eluent.

20) Preparation of 4-hydroxychalcone



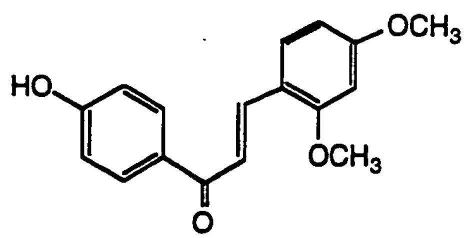
2.2 g (18 mmol) of acetophenone and 1.8 g (15 mmol) of 4-hydroxybenzaldehyde were added to a solution of 12 g of sodium hydroxide in 10 ml of water and 5 ml of ethanol. The solution was refluxed for 1 h and left at room temperature for 3 days. The reaction mixture was acidified with 70 ml of 4 M hydrochloric acid and filtered to give 3.78 g of yellow crystals which were recrystallized from ethanol to give 1.85 g of 4-hydroxychalcone identical to that described by R. L. Shriner and T. Kurosawa in *J. Am. Chem. Soc.* 52 (1930), 2538-2540.

21) Preparation of 2,4-dimethoxy-4'-prop-2-enyloxychalcone



0.88 g of 4-allyloxyacetophenone and 0.42 g of 2,4-dimethoxybenzaldehyde were dissolved in 3.5 ml of ethanol, and to the solution was added ethanol saturated with 3.5 ml of hydrogen chloride. The mixture was left at room temperature for 30 min during which time it turned heavily red. The solution was concentrated *in vacuo* and the residue chromatographed (column chromatography over silica gel 60 (Merck 0.063-0.200 mm, 100 g), eluent toluene and toluene to which increasing amounts of ethyl acetate were added) to give 500 mg of 4-methoxyacetophenone and 471 mg of 2,4-dimethoxy-4'-prop-2-enyloxychalcone. The chalcone was recrystallized from petroleum ether-ethyl acetate to give 370 mg of slightly yellow crystals. For data, see Example 2.24.

23) Preparation of 2,4-dimethoxy-4'-hydroxychalcone



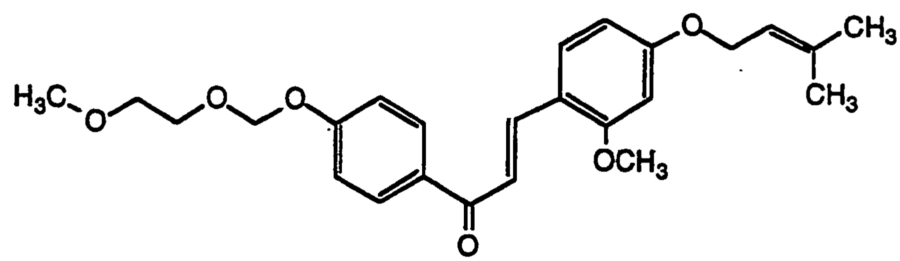
326 mg of 2,4-dimethoxy-4'-allyloxychalcone was dissolved in 5 ml of methanol, 1 ml of added water, palladium on charcoal (10%, 100 mg), and 100 mg of p-toluene-sulfonic acid, and the mixture was refluxed for 24 h. The reaction mixture was filtered and poured into a mixture of 5 ml of a 10% aqueous solution of sodium bicarbonate and 5 ml of a saturated aqueous sodium chloride solution. The solution was extracted with 10 ml of ethyl acetate and concentrated to give 380 mg which were chromatographed over silica gel 60 (Merck 0.063-0.200 mm, 25 g, eluent petroleum ether-ethyl acetate 9:1) to give 130 mg of yellow crystals which were recrystallized from methanol to give 70 mg of 2,4-dimethoxy-4'-hydroxychalcone, m.p. 165-166°C.

^{1}H-NMR data (200 MHz, CD_3CN-DMSO-d_6, δ): 7.98 (AA-part of an AA'MM'-system, H-2'and H-6'), 7.96 (d, *J* 15 Hz; H-β), 7.72 (d, *J* 7 Hz, H-6), 7.65 (d, *J* 15 Hz, H-α), 6.91 (MM'-part of an AA'MM'-system, H-3'and H-5'), 6.59 (dd, *J* 7 and 2 Hz, H-5), 6.57 (d, *J* 2 Hz, H-3), 3.90 and 3.83 (CH_3-O).

^{13}C NMR data (50 MHz, CD_3CN-DMSO-d_6, δ) 187.5, 138.8, 157.8, 117.5, 161.1, 99.2, 162.9, 106.8, 13.0, 131.7, 116.2, 164.0, 116.2, 131.7, 56.4, 56.2.

Calc. for $C_{17}H_{16}O_4$ C 71.82, H 5.67. Found: C 71.48, H 5.82.

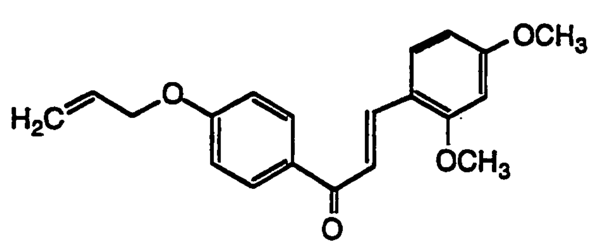
23) Preparation of 4'-(2-methoxyethoxymethoxy)-4-(3-methylbut-2-enyloxy)-2-methoxy-chalcone



0.9 g (4.1 mmol) of 4-(2-methoxyethoxymethoxy)acetophenone, 1.77 g (7.9 mmol) of 4-(3-methyl)but-2-enyloxy-2-methoxybenzaldehyde, and 4 g of potassium hydroxide were dissolved in 16 ml of ethanol, and the solution was left for 24 h. The solution was poured into 20 ml of 4 M hydrochloric acid and extracted twice with 10 ml of ether. The ether phase was concentrated *in vacuo* to give 2.7 g of a yellow oil which was purified by column chromatography (silica gel 60 (Merck 0.063-0.200 mm, 250 g), eluent toluene-ethyl acetate 9:1, to which increasing amounts of ethyl acetate were added). 4'-(2-Methoxyethoxymethoxy)4-(3-methylbut-2-enyloxy)-2-methoxychalcone was obtained as an oil (860 mg, 49%).

^{1}H NMR data (200 MHz, CD_3CN, δ) 8.05 (AA'-part of an AA'MM'-system, H-2' and H-6'), 7.98 (d, *J* 15 Hz, H-β), 7.68 (d, *J* 8 Hz, H-6), 7.60 (d, *J* 15 Hz, H-α), 7.12 (MM'-part of an AA'MM'-system, H-3' and H-5'), 6.55 (dd, *J* 3 and 8 Hz, H-4), 6.53 (d, *J* 3 Hz, H-3), 5.45 (m, =CH-), 5.32 (O-CH_2-O), 4.61 (d, CH_2-C= *J* 7 Hz), 3.90 (s, CH_3O-Ar), 3.80 (AA'-part of an AA'MM'-system, CH_2-O-Ar), 3.55 (MM'-part of an AA'MM'-system, C-O-CH_2-), 3.26 (s, CH_3-O-C-C), 1.78 and 1.73 (s, CH_3-C).

24) Preparation of 2,4-dimethoxy-4'-prop-2-enyloxychalcone



17.6 g (0.1 mol) of 4-allyloxyacetophenone and 16.6 g (0.1 mol) of 2,4-dimethoxybenzaldehyde were under an inert dry atmosphere (argon) dissolved in 100 ml of dry

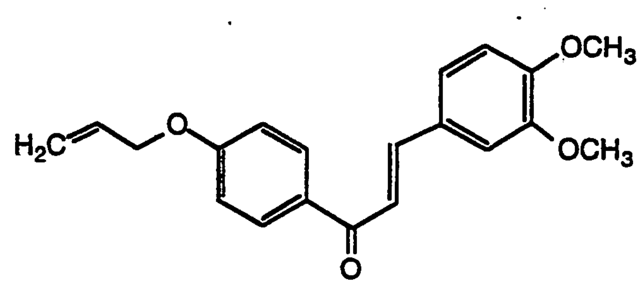
ethanol (freshly distilled from sodium under argon atmosphere). The solution was added 1 g of sodium hydroxide and left under stirring for 18 h. The reaction mixture was filtered to give 29.9 g (97 %) of 2,4-dimethoxy-4'-prop-2-enyloxychalcone identical to that obtained in Example 2.22, m.p. 74.5-75°C.

^{1}H NMR data (200 MHz, $CDCl_3$, δ) 8.08 (d, *J* 16 Hz, H-β), 8.03 (AA'-part of an AA'MM'-system, H-2' and H-6'), 7.56 (d, *J* 16 Hz, H-α), 7.56 (d, *J* 8 Hz, H-6), 6.98 (MM'-part of an AA'MM'-system, H-3' and H-5'), 6.51 (dd, *J* 3 and 8 Hz, H-5), 6.45 (d, *J* 3 Hz, H-3), 6.03 (ddt, *J* 15, 10 and 4 Hz, -C$\underline{H}$=), 5.41 (d, *J* 15 Hz, =CH$\underline{H}$), 5.28 (d, *J* 10 Hz, =C$\underline{H}$H), 4.59 (d, *J* 4 Hz, -CH_2-), 3.89 and 3.85 (s, CH_3O).

^{13}C NMR data (50 MHz, $CDCl_3$, δ) 189.3, 162.9, 162.0, 160.3, 139.6, 132.6, 131.7, 130.8, 130.6, 120.0, 118.0, 117.2, 114.4, 105.4, 98.4, 68.8, 55.5, 55.4.

Calc. for $C_{20}H_{20}O_4$: C 74.06, H 6.21. Found: C 74.12, H 6.30

25) Preparation of 3,4-dimethoxy-4'-prop-2-enyloxychalcone



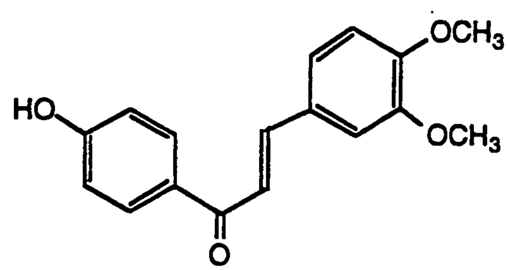
1.76 g (10 mmol) of 4-allyloxyacetophenone and 1.66 g (10 mmol) of 3,4-dimethoxybenzaldehyde were under a dry inert atmosphere (argon) dissolved in 10 ml dry ethanol and the solution was stirred for 18 h. The solution was filtered to give 3.0 g (99 %) of 3,4-dimethoxy-4'-prop-2-enyloxychalcone which was recrystallized from ethanol, m.p. 74.5-75°C.

^{1}H NMR data (200 MHz, CD_3CN, δ) 8.06 (AA'-part of an AA'MM'-system, H-2' and H-6'), 7.70 (d, *J* 15 Hz, H-β), 7.58 (d, *J* 15 Hz, H-α), 7.33 (d, *J* 2 Hz, H-2), 7.25 (dd, *J* 2 and 8 Hz, H-6), 7.00 (MM'-part of an AA'MM'-system, H-3' and H-5'), 6.91 (d, *J* 8 Hz, H-5), 6.04 (m, =C$\underline{H}$-), 5.42 (m, =CH$\underline{H}$), 5.28 (m, =C$\underline{H}$H), 4.60 (m, -CH_2-), 3.87 and 3.83 (s, CH_3).

^{13}C NMR data (50 MHz, CD_3CN, δ) 188.3, 161.8, 151.0, 148.9, 142.9, 132.7, 130.8, 130.1, 127.4, 122.8, 119.1, 116.8, 113.9, 110.9, 109.9, 68.1, 54.9, 54.8.

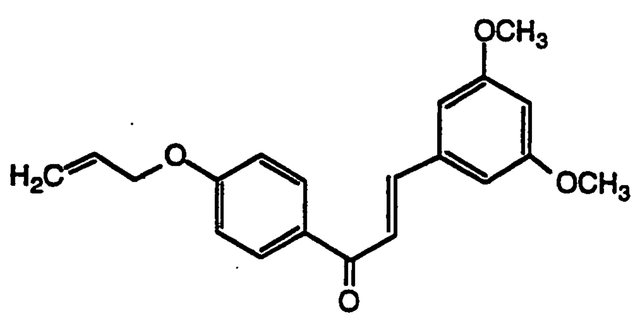
Calc. for $C_{20}H_{20}O_4$: C 74.06, H 6.21. Found: C 74.10, H 6.24.

26) Preparation of 3,4-dimethoxy-4'-hydroxychalcone



486 mg (1.5 mmol) of 3,4-dimethoxy-4'-allyloxychalcone was dissolved in a mixture of 7.5 ml of methanol and 1.5 ml of water. To the solution were added 150 mg of p-toluenesulfonic acid and 150 mg of palladium on carbon (10 %) and the solution was heated to 80°C for 2 h in a sealed flask. The reaction mixture was filtered and poured into a mixture of 10 ml of an aqueous 10% solution of sodium bicarbonate and 10 ml of a saturated aqueous solution of sodium chloride. The mixture was extracted with 15 ml of ethyl acetate and concentrated *in vacuo*. The residue was recrystallized from methanol to give 0.125 g (25%) of 3,4-dimethoxy-4'-hydroxychalcone (m.p. 193-198°C) identical to that described by A.v.n Wacek, and E. David, *Ber.* 70 (1937), 190 (m.p. 208°C).

27) Preparation of 3,5-dimethoxy-4'-prop-2-enyloxychalcone



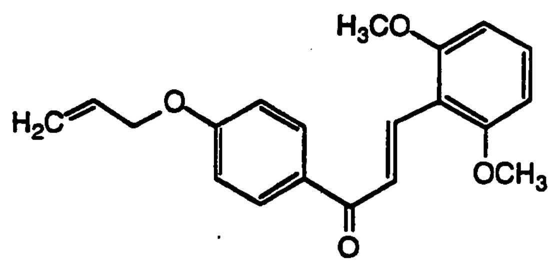
1.76 g (10 mmol) of 4-allyloxyacetophenone and 1.66 g (10 mmol) of 3,5-dimethoxybenzaldehyde were dissolved in 10 ml of dry freshly distilled ethanol under an inert atmosphere (argon), and the solution was admixed with 100 mg of sodium hydroxide and left under stirring for 18 h. The reaction mixture was filtered to give 2.96 g (99 %) of 3,5-dimethoxy-4'-prop-2-enyloxychalcone which was recrystallized from methanol, m.p. 88.5-90°C.

^1H NMR data (200 MHz, CD_3CN, δ) 8.06 (AA´-part of an AA´MM´-system, H-2´ and H-6´), 7.68 (d, *J* 15 Hz, H-β), 7.62 (d, *J* 15 Hz, H-α), 7.01 (MM´-part of an AA´MM´-system, H-3´and H-5´) 6.88 (d, *J* 2 Hz, H-2 and H-6) 6.53 (t, *J* 2 Hz, H-4), 6.10 (m, =C<u>H</u>-), 5.39 (m, =CH<u>H</u>), 5.28 (m, =C<u>H</u>H), 4.61 (m, -CH_2-), 3.80 (s, CH_3O).

^{13}C NMR data (50 MHz, CD_3CN, δ): 187.4, 161.9, 160.6, 142.6, 136.6, 132.6, 130.5, 130.3, 122.0, 116.8, 113.9, 116.8, 113.9, 105.8, 101.9, 68.2, 54.7.

Calc. for $C_{20}H_{20}O_4$: C 74.06, H 6.21. Found: C 74.02, H 6.24

28) <u>Preparation of 2,6-dimethoxy-4'-prop-2-enyloxychalcone</u>



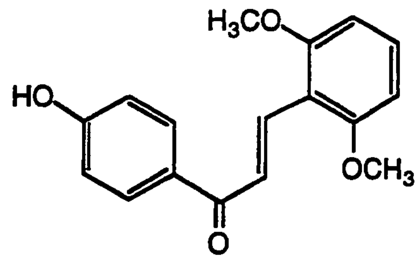
0.59 g (3.3 mmol) of 4-allyloxyacetophenone and 0.56 g (3.3 mmol) of 2,6-dimethoxybenzaldehyde were under an inert atmosphere (argon) dissolved in 3.3 ml of dry freshly distilled ethanol and 100 mg of sodium hydroxide was added to the solution. The mixture was left with stirring for 4.5 h, poured into 10 ml of M hydrochloric acid and extracted with 10 ml of ethyl acetate. The organic phase was dried over $MgSO_4$ and concentrated *in vacuo* to give 0.89 g of a yellow gum, from which 2,6-dimethoxy-4'-prop-2-enyloxychalcone (0.70 g, 70%) was isolated by column chromatography over silica gel 60 (Merck 0.063-0.200 mm, 80 g) using petroleum ether-ethyl acetate (2:1, 800 ml) with 0.5% added glacial acetic acid as an eluent. Crystallization from methanol afforded 0.56 g of 2,6-dimethoxy-4'-prop-2-enyloxychalcone, m.p. 102-103°C.

^1H NMR data (200 MHz, $CDCl_3$, δ) 8.26 (d, *J* 15 Hz, H-β), 8.03 (AA'-part of an AA'MM'-system, H-2' and H-6'), 7.99 (d, *J* 15 Hz, H-α), 7.27 (t, *J* 7 Hz, H-4), 6.98 (MM'-part of an AA'MM'-system, H-3' and H-5'), 6.57 (d, *J* 7 Hz, H-3 and H-5), 6.05 (m, =CH-), 5.42 (m, =CH<u>H</u>), 5.32 (m, =C<u>H</u>H), 4.60 (m, -CH_2-), 3.90 (s, CH_3-O).

^{13}C NMR data (50 MHz, $CDCl_3$, δ): 190.0, 161.6, 159.9, 134.6, 132.3, 131.7, 130.9, 130.4, 124.4, 117.7, 114.0, 112.7, 103.4, 68.5, 55.5.

Calc. for $C_{20}H_{20}O_4$: C 74.06, H 6.21. Found: C 73.79, H 6.34

29) Preparation of 2,6-dimethoxy-4'-hydroxychalcone

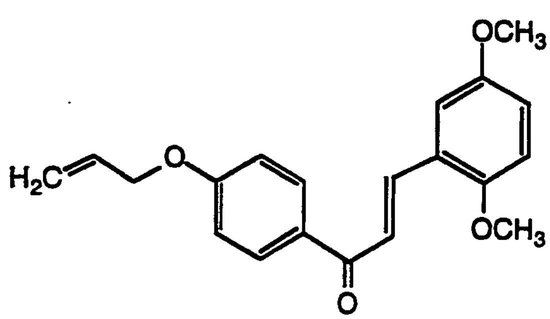


0.32 g (1 mmol) of 2,6-dimethoxy-4'-prop-2-enyloxychalcone was dissolved in 5 ml of methanol, and to the solution was added 1 ml of water, 0.6 g of 10% of palladium on carbon, and 0.1 g of paratoluenesulfonic acid. The mixture was refluxed for 2 h and filtered, and the filtrate was poured into 5 ml of water. The solution was concentrated *in vacuo* to half the volume and poured into 12 ml of ethyl acetate. The organic phase was washed with 10 ml of a saturated aqueous solution of sodium hydrogen carbonate and subsequently with 10 ml of a saturated aqueous solution of sodium chloride and concentrated *in vacuo* to give 65.1 mg of a yellow gum. The gum was chromatographed over silica gel 60 (Merck 0.063- 0.200 mm, 10 g) using petroleum ether-ethyl acetate (2:1) added 0.5 % of acetic acid as an eluent to give 2,6-dimethoxy-4'-hydroxychalcone, which was crystallized from methanol to give 23 mg (7%) of yellow crystals, m.p. 172-176°C.

^{1}H NMR data (200 MHz, $CDCl_3$, δ) 8.21 (d, *J* 15 Hz, H-β), 7.92 (d, *J* 15 Hz, H-α), 7.92 (AA'-part of an AA'MM'-system, H-2' and H-6'), 7.30 (t, *J* 7 Hz, H-4), 6.91 (MM'-part of an AA'MM'-system, H-3' and H-5'), 6.58 (d, *J* 7 Hz, H-3 and H-5), 3.90 (s, CH_3-O).

Calc. for $C_{17}H_{16}O_4$: C 71.82, H 6.57. Found: C 71.98, H 5.81.

30) Preparation of 2,5-dimethoxy-4'-prop-2-enyloxychalcone



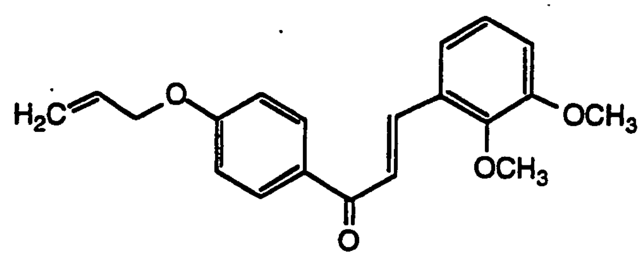
1.76 g (10 mmol) of 4-allyloxyacetophenone and 1.66 g (10 mmol) of 2,5-dimethoxybenzaldehyde were under an inert atmosphere (argon) dissolved in 10 ml of dry freshly distilled ethanol, and 100 mg of sodium hydroxide was added to the solution. The mixture was left under stirring for 2.5 h and filtered. The precipitate was recrystallized from methanol to give 2.50 g (83%) of 2,5-dimethoxy-4'-prop-2-enyloxychalcone, m.p. 86-87°C.

^{1}H NMR data (200 MHz, $CDCl_3$, δ) 8.06 (d, J 15 Hz, H-β), 8.02 (AA'-part of an AA'MM'-system, H-2' and H-6'), 7.59 (d, J 15 Hz, H-α), 7.16 (d, J 2 Hz, H-6), 6.97 (MM'-part of an AA'MM'-system, H-3' and H-5'), 6.93 (dd, J 2 and 7 Hz, H-4), 6.84 (d, J 7 Hz, H-3), 6.05 (m, =CH-), 5.42 (m, =CH<u>H</u>), 5.32 (m, =C<u>H</u>H), 4.60 (m, $-CH_2-$), 3.84 (s, CH_3-O), 3.80 (s, CH_3-O).

^{13}C NMR data (50 MHz, $CDCl_3$, δ) 189.1, 162.3, 153.5, 153.2, 129.3, 132.6, 131.4, 130.8, 124.7, 122.8, 118.2, 116.9, 114.5, 113.8, 112.4, 88.9, 56.1, 55.8.

Calc. for $C_{20}H_{20}O_4$: C 74.06, H 6.21. Found: C 73.81, H 6.18.

31) <u>Preparation of 2,3-dimethoxy-4'-prop-2-enyloxychalcone</u>



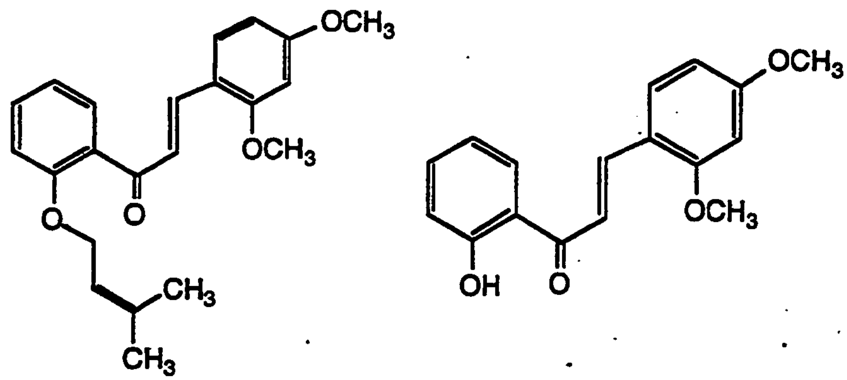
1.76 g (10 mmol) of 4-allyloxyacetophenone and 1.66 g (10 mmol) of 2,3-dimethoxybenzaldehyde were under an inert atmosphere (argon) dissolved in 10 ml of dry freshly distilled ethanol, and 100 mg of sodium hydroxide was added to the solution. The mixture was left under stirring for 23 h and concentrated *in vacuo*. The crystalline residue was recrystallized from methanol-water to give 2.92 g (90%) of 2,3-dimethoxy-4'-prop-2-enyloxychalcone, m.p. 98-99°C.

^{1}H NMR data (200 MHz, $CDCl_3$, δ) 8.09 (d, J 15 Hz, H-β), 8.03 (AA'-part of an AA'MM'-system, H-2' and H-6'), 7.59 (d, J 15 Hz, H-α), 7.27 (d, J 2 Hz, H-6), 7.09 (t, J 7 Hz, H-5), 6.96 (MM'-part of an AA'MM'-system, H-3' and H-5'), 6.95 (dd, J 2 and 7 Hz, H-4), 6.05 (m, =CH-), 5.42 (m, =CH<u>H</u>), 5.32 (m, =C<u>H</u>H), 4.60 (m, $-CH_2-$), 3.88 (s, CH_3-O), 3.46 (s, CH_3-O).

^{13}C NMR data (50 MHz, $CDCl_3$, δ) 189.1, 162.4, 153.2, 148.8, 138.9, 132.5, 131.2, 130.8, 129.2, 124.2, 123.4, 119.6, 118.2, 114.6, 114.4, 69.0, 61.3, 55.9.

Calc. for $C_{20}H_{20}O_4$ C 74.06, H 6.21. Found: C 73.99, H 6.24

32) Preparation of 2,4-dimethoxy-2'-(3-methylbut-2-enyloxy)chalcone and 2,4-dimethoxy-2'-hydroxychalcone



1.76 g (10 mmol) of 2-(3-methylbut-2-enyloxy)acetophenone and 1.66 g (10 mmol) of 2,4-dimethoxybenzaldehyde were under an inert atmosphere (argon) dissolved in 10 ml of dry freshly distilled ethanol, and to the solution was added 100 mg of sodium hydroxide. The mixture was left under stirring for 4.5 h, poured into 10 m l of 1 M hydrochloric acid and extracted with 10 ml of ethyl acetate. The organic phase was dried over $MgSO_4$, and concentrated *in vacuo* to give 3.2 g of a yellow gum, from which 2,4-dimethoxy-2'-hydroxychalcone (0.85 g, 27%) and 2,4-dimethoxy-2'-(3-methylbut-2-enyloxy)chalcone (1.24 g, 40%) was isolated by column chromatography over silica gel 60 (Merck 0.063-0.200 mm, 325 g) using toluene-ethyl acetate (19:1, 1000 ml) with 0.5% added glacial acetic acid and toluene-ethyl acetate (14:1, 1000 ml) with 0.5% added glacial acetic acid as eluents. Crystallization of 2,4-dimethoxy-2'-hydroxychalcone from methanol afforded 0.42 g of 2,4-dimethoxy-2'-(3-methylbut-2-enyloxy)chalcone.

2,4-Dimethoxy-2'-hydroxychalcone has previously been reported used for studies on oxidation of chalcones with lead tetraacetate, see K. Kurosawa, and J. Higuchi in *J. Bull. Soc. Japan* 45 (1972), 1132-1136, and K. Kurosawa in *J. Bull. Chem. Soc. Japan* 42 (1969), 1456.

^{1}H NMR data of 2,4-dimethoxy-2'-hydroxychalcone (200 MHz, $CDCl_3$, δ) 8.15 (d, *J* 15

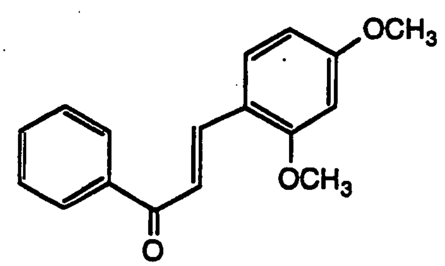
Hz, H-β), 7.89 (dd, *J* 2 and 8 Hz, H-6') 7.66 (d, *J* 15 Hz, H-α) 7.56 (d, *J* 7 Hz, H-6), 7.46 (dt, *J* 2 and 7 Hz, H-4'), 6.99 (dd, *J* 2 and 7 Hz, H-3'), 6.88 (dt, *J* 2 and 7 Hz, H-5'), 6.53 (dd, *J* 2 and 7 Hz, H-5) 6.46 (d, *J* 2 Hz, H-3), 3.90 (s, CH_3-O), 3.89 (s, CH_3-O).

^{13}C NMR data of 2,4-dimethoxy-2'-hydroxychalcone (50 MHz, $CDCl_3$, δ) 194.2, 163.5, 163.4, 160.7, 141.3, 135.8, 131.5, 129.9, 120.3, 118.6, 118.4, 118.0, 116.2, 105.6, 98.4, 55.6, 55.5.

^1H NMR data of 2,4-dimethoxy-2'-(3-methylbut-2-enyloxy)chalcone (200 MHz, $CDCl_3$, δ) 7.92 (d, *J* 15 Hz, H-β), 7.64 (dd, *J* 2 and 8 Hz, H-6'), 7.51 (d, *J* 7 Hz, H-6), 7.42 (d, *J* 15 Hz, H-α) 7.39 (dt, *J* 2 and 7 Hz, H-4'), 7.00 (dt, *J* 2 and 7 Hz, H-5'), 6.97 (dd, *J* 2 and 7 Hz, H-3'), 6.49 (dd, *J* 2 and 7 Hz, H-5) 6.44 (d, *J* 2 Hz, H-3), 5.48 (m, =CH-) 4.60 (d, *J* 6 Hz, CH_2), 3.84 (s, CH_3-O), 3.83 (s, CH_3-O), 1.74 (s, CH_3-C), 1.70 (s, CH_3-C).

^{13}C NMR data of 2,4-dimethoxy-2'-(3-methylbut-2-enyloxy)chalcone (50 MHz, $CDCl_3$, δ) 193.3, 162.7, 160.1, 157.5, 138.3, 132.4, 130.5, 130.0, 125.4, 120.7, 119.7, 113.1, 105.4, 98.3, 65.7, 55.5, 25.7, 18.3.

33) Preparation of 2,4-dimethoxychalcone



1.20 g (10 mmol) of acetophenone and 1.66 g (10 mmol) of 2,4-dimethoxybenzaldehyde were dissolved in 10 ml of dry freshly distilled ethanol under an inert atmosphere (nitrogen or argon), and 100 mg of sodium hydroxide was added to the solution. The mixture was left under stirring for 23 h, poured into 10 ml of 1 M hydrochloric acid and extracted with 10 ml of ethyl acetate. The organic phase was dried over $MgSO_4$ and concentrated *in vacuo* to give 2.5 g of a yellow gum, from which 2.25 g of 2,4-dimethoxychalcone was isolated by column chromatography over silica gel 60 (Merck 0.063-0.200 mm, 250 g) using petroleum ether-ethyl acetate (14:1, 1700 ml) with 0.5% added glacial acetic acid and petroleum ether-ethyl acetate (9:1, 900 ml) with 0.5% added glacial acetic acid as eluents. Crystallization from methanol-water afforded 0.73 g (25%) of 2,4-dimethoxychalcone, m.p. 49-50°C.

2,4-Dimethoxychalcone has previously been used for chemical studies, see V. F. Laurushin, N. D. Trusevich, and V. N. Tolmachev in *Zh. Obshch Khim.* 39 (1969), 42-

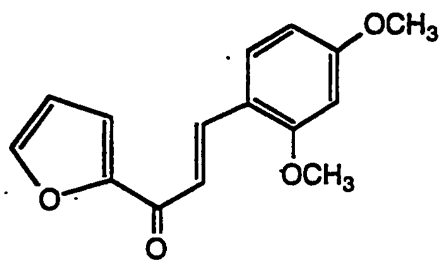
45, *Chem. Abstr.* 70 (1969), 105822t, and A. M. Volovick, V. N. Tolmachev, and V. F. Laurushin in *Visn Kharkiv Univ. Khim.* 73 (1971), 85-88, *Chem. Abstr.* 78 (1973), 57241u.

^{1}H NMR data (200 MHz, $CDCl_3$, δ) 8.06 (d, *J* 15 Hz, H-β), 8.02-7.95 (m, H-2' and H-6'), 7.53 (d, *J* 15 Hz, H-α), 7.6-7.4 (m, H-6 and H-3'-5'), 6.51 (dd, *J* 2 and 8 Hz, H-5), 6.45 (d, *J* 2 Hz, H-3), 3.87 (s, CH_3-O), 3.82 (s, CH_3-O).

^{13}C NMR data (50 MHz, $CDCl_3$, δ) 191.1, 163.0, 160.4, 140.5, 132.3, 130.9, 128.4, 128.4, 120.3, 117.1, 105.4, 98.4, 55.5, 55.4.

Calc. for $C_{17}H_{16}O_3$: C 76.10, H 6.01. Found: C 76.17, H 6.09.

34) Preparation of 1-(furan-2-yl)-3-(2,4-dimethoxyphenyl)prop-2-en-1-on



1.09 g (10 mmol) of 2-acetylfurane and 1.66 g (10 mmol) 2,4-dimethoxybenzaldehyde were under an inert atmosphere (argon) dissolved in 10 ml of dry freshly distilled ethanol, and 100 mg of sodium hydroxide was added to the solution. The mixture was left under stirring for 2.5 h, poured into 45 ml of 1 M hydrochloric acid and extracted with 45 ml of ethyl acetate. The organic phase was dried over $MgSO_4$, and concentrated *in vacuo* to give 2.5 g of a yellow gum, from which 2.0 g (80%) of 1-(furan-2-yl)-3-(2,4-dimethoxyphenyl)prop-2-en-1-on was isolated as a yellow oil by column chromatography over silica gel 60 (Merck 0.063-0.200 mm, 240 g) using petroleum ether-ethyl acetate (8:2, 2500 ml) with 0.5% added glacial acetic acid as an eluent. The compound crystallized upon standing, m.p. 57-59°C (MeOH-H_2O).

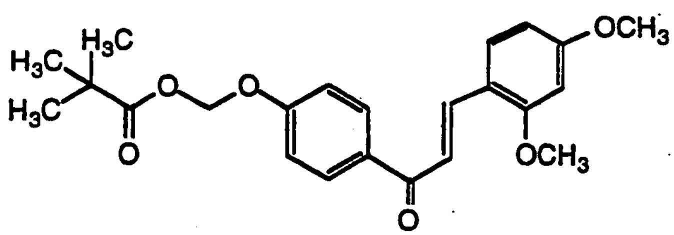
^{1}H NMR data (200 MHz, $CDCl_3$, δ) 8.12 (d, *J* 15 Hz, H-β), 7.62 (dd, *J* 1 and 2 Hz, H-5'), 7.55 (d, *J* 7Hz, H-6), 7.53 (d, *J* 15 Hz, H-α), 7.29 (dd, *J* 1 and 3 Hz, H-3'), 6.55 (dd, *J* 2 and 3 Hz, H-4'), 6.51 (dd, *J* 2 and 7 Hz, H-5), 6.44 (d, *J* 2 Hz, H-3), 3.86 (s, CH_3-O), 3.81 (s, CH_3-O).

^{13}C NMR data (50 MHz, $CDCl_3$, δ) 178.8, 163.2, 160.5, 154.0, 146.3, 139.7, 130.9, 119.2,

117.1, 116.8, 112.4, 105.6, 98.3, 55.5, 55.4.

Calc. for $C_{15}H_{14}O_4$: C 69.76, H 5.46. Found: C 69.88, H 5.61.

35) Preparation of 2,4-dimethoxy-4'-pivaloyloxymethoxychalcone

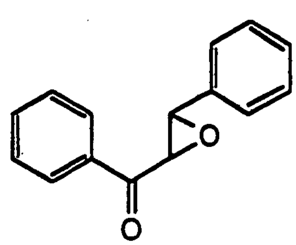


An acetonic solution of iodomethyl pivalate was prepared by allowing 0.29 g (2.1 mmol) of chloromethylpivalate to react for 30 min with 0.15 g (5.7 mmol) of sodium iodide dissolved in 10 ml of dry acetone. The acetonic solution was decanted from the precipitated sodium chloride and added to a suspension of 0.57 g (2 mmol) of 2,4-dimethoxy-4'-hydroxychalcone and 0.5 g (3.7 mmol) of potassium carbonate, which had previously been stirred under argon atmosphere for 30 min. The combined mixtures were left for 2 days at 40°C in a sealed flask, filtered, and concentrated *in vacuo* to give a yellow gum, from which 0.48 g (60%) of 2,4-dimethoxy-4'-pivaloyloxy-methoxychalcone was isolated as a yellow oil by column chromatography over silica gel 60 (Merck 0.063-0.200 mm, 80 g) using petroleum ether-ethyl acetate (9:1, 1500 ml) as an eluent. The compound crystallized upon standing, m.p. 98-99°C (methanol).

^{1}H NMR data (200 MHz, $CDCl_3$, δ) 8.09 (d, *J* 15 Hz, H-β), 8.05 (AA'-part of an AA'MM'-system, H-2' and H-6'), 7.57 (d, *J* 7 Hz, H-6), 7.54 (d, *J* 15 Hz, H-α), 7.10 (MM'-part of an AA'MM'-system, H-3'and H-5'), 6.53 (dd, *J* 2 and 7 Hz, H-15), 6.47 (d, *J* 2 Hz, H-3), 5.82 (s, CH_2), 3.86 (s, CH_3-O), 3.85 (s, CH_3-O), 1.21 (s, CH_3-C).

Calc. for $C_{23}H_{26}O_6$ C 69.33, H 6.58. Found: C 69.29, H 6.56

36) Preparation of chalcone epoxide



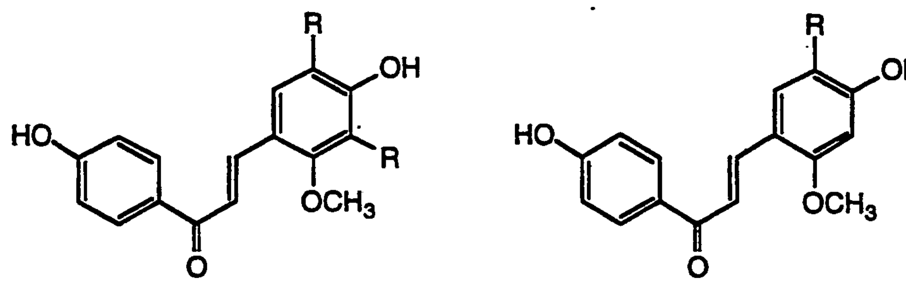
1.040 g (5 mmol) of chalcone was dissolved in 15 ml of ethanol and to the solution was added 2 ml of 25% hydrogen peroxide and 2 ml of an aqueous solution of 1 M sodium carbonate. The precipitate which was formed after stirring for 3 h was isolated and recrystallized from DMSO-water to give 250 mg (22%) of chalcone epoxide.

^{1}H NMR data (200 MHz, DMSO-d_6, δ) 8.04 (d, *J* 8 Hz, H-2' and H-6'), 7.9-7.3 (complex pattern, H-2-6 and H-3'-5'), 4.85 (broad s, H-α), 4.18 (H-β).

^{13}C NMR data (50 MHz, DMSO-d_6, δ) 192.7, 156.8, 143.9, 133.7, 128.7, 128.6, 128.2, 127.9, 126.1, 59.6, 58.2.

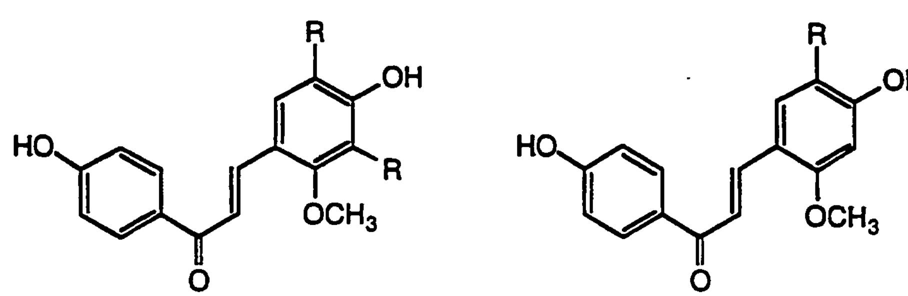
Calc. for $C_{15}H_{12}O_2$: C 80.34, H 5.39. Found: C 80.25, H 5.37.

37) <u>Preparation of 2-methoxy-5-alkyl-4,4'-dihydroxychalcones or 2-methoxy-3,5-dialkyl-4,4'-dihydroxychalcones</u>



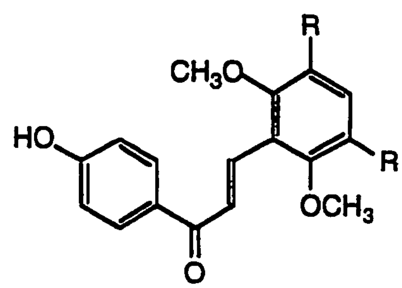
2-Methoxy-4-alk-2-enyloxybenzaldehyde is condensed with an 4-alk-2-enyloxyacetophenone as described in the synthesis of the chalcone allyl ethers (see e.g. Example 2.24). The protecting alkenyl groups are removed by heating an acidic methanolic solution to which is added a small amount of water with palladium on carbon as described in combination with the synthesis of 4'-hydroxychalcone (Example 2.19).

38) <u>Preparation of 2-methoxy-5-alkyl-4,4'-dihydroxychalcones or 2-methoxy-3,5-dialkyl-4-4'-dihydroxychalcones</u>



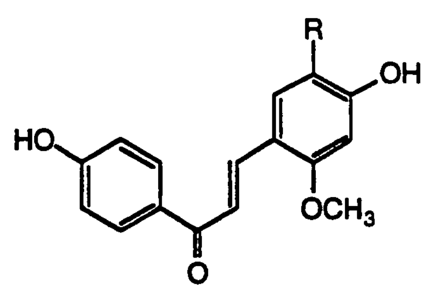
The appropriate 2-methoxy-5-alkyl-4-hydroxybenzaldehyde or 2-methoxy-3,5-dialkyl-4-hydroxybenzaldehyde is condensed with 4-hydroxyacetophenone in acidic ethanol as described in combination with the synthesis of licochalcone A (Example 2.16).

39) Preparation of 2-methoxy-3,5-dialkyl-6,4'-dihydroxychalcones



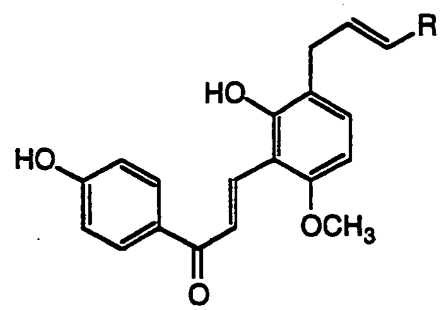
The appropriate 2-methoxy-3,5-dialkyl-6-hydroxybenzaldehyde is condensed with 4-hydroxyacetophenone in alkaline aqueous ethanol as described by T. A. Geissman and R. O. Clinton in *J. Am. Chem. Soc.* **68** (1946), 697-700.

40) Preparation of 2-methoxy-5-alk-2-enyl-4,4'-dihydroxychalcones



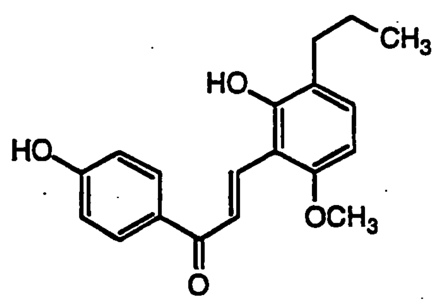
The appropriate 2-methoxy-5-alk-2-enyl-4-hydroxybenzaldehyde is condensed with 4-hydroxyacetophenone as described in the synthesis of licochalcone A (Example 2.16).

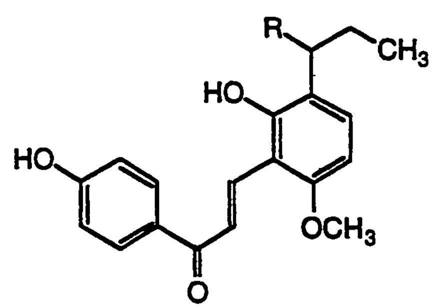
41) Preparation of 2-methoxy-5-alk-2-enyl-6,4'-dihydroxychalcones

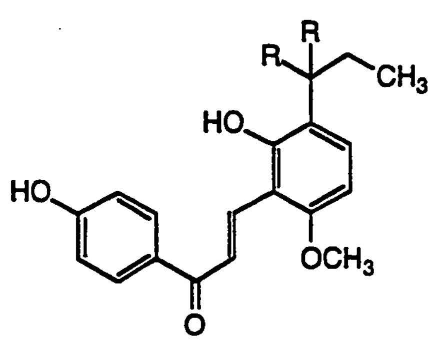


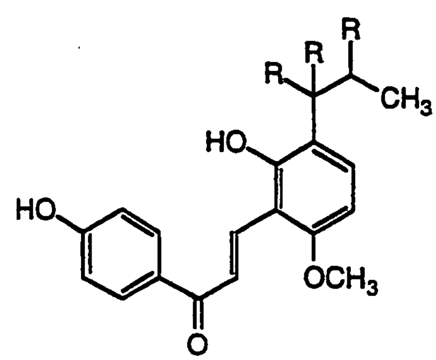
The appropriate 2-methoxy-5-alk-2-enyl-6-hydroxybenzaldehyde is condensed with 4-hydroxyacetophenone as described by T. A. Geissman and R. O. Clinton in *J. Am. Chem. Soc.* 68 (1946), 697-700.

42) Preparation of 2-methoxy-5-propyl-6,4'-dihydroxychalcone, 2-methoxy-5-(α-alkylpropyl)-6,4'-dihydroxychalcones, 2-methoxy-5-(α,α-dialkylpropyl)-6,4'-dihydroxy-chalcones or 2-methoxy-(α,α,β-dialkylpropyl)-6,4'-dihydroxychalcones



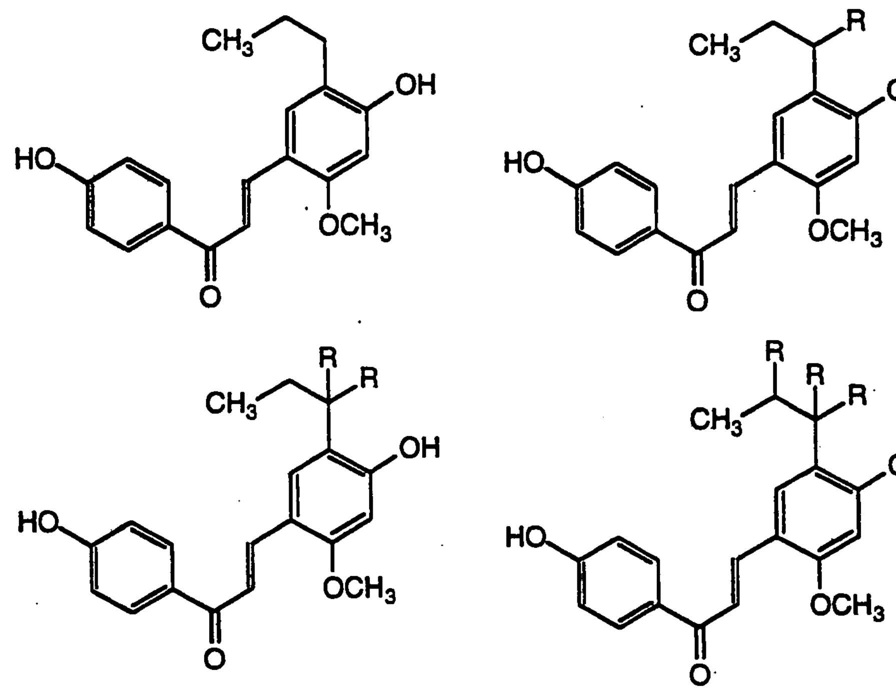






A solution of the appropriate 2-methoxy-5-alk-2-enyl-4,4'-dihydroxychalcone (e.g. licochalcone A) in an aprotic solvent is hydrogenated using dyrdidocarbonyltris(triphenylphosphine)rhodium(I) as a catalyst. This catalyst selectively catalyses the reduction of terminal double bonds.

43) Preparation of 2-methoxy-5-propyl-4,4'-dihydroxychalcone, 2-methoxy-5-(α-alkylpropyl)-4,4'-dihydroxychalcones, 2-methoxy-5-(α,α-dialkylpropyl)-4,4'-dihydroxychalcone or 2-methoxy-(α,α,β-dialkylpropyl)-4,4'-dihydroxychalcones



A solution of the appropriate 2-methoxy-5-alk-2-enyl-4,4'-dihydroxychalcone (e.g. licochalcone A) in an aprotic solvent is hydrogenated using hydridocarbonyltris-(triphenylphosphine)rhodium(I) as a catalyst which selectively catalyses the reduction of terminal double bonds.

EXAMPLE 3

Formulations of pharmaceutical compositions

Tablets

An appropriate amount of α,β-unsaturated bis-aromatic ketone is added to a mixture of potato starch and lactose (7:3) and a granule is prepared by moistening with a 4% solution of gelatin in water, sifting and drying. Tablets are compressed after addition of glidants and lubricants such as magnesium stearate and talc.

Thus, tablets containing 500 mg of α,β-unsaturated bis-aromatic ketone are prepared by mixing 500 g of the ground chalcone with 36 g of lactose and 84 g of potato starch. The mixture is moistened with a 4% aqueous solution of gelatin and converted into a granulate by sifting or spraying. The granulate is compressed into 1000 tablets with an average weight of 650 mg and a diameter of 13.5 mm after addition of 30 g of a mixture of talc and magnesium stearate (9:1).

Suppositories

The appropriate amount of α,β-unsaturated bis-aromatic ketone is suspended into 2 g of melted hard fat and poured into a matrix.

Capsules

The appropriate amount of α,β-unsaturated bis-aromatic ketone, if convenient mixed with a diluent like potato starch, lactose or both, is filled into a prefabricated cylindrical capsule, and the capsule is closed.

Liquids for oral administration

The α,β-unsaturated bis-aromatic ketone is dissolved in a mixture of water and ethanol. Flavouring substances such as a licorice extract and a sugar solution may be added to the solution.

EXAMPLE 4

Effect of licochalcone A on the *in vitro* growth of *L. major* and *L. donovani* promastigotes

Materials and methods

Parasite cultures. The WHO reference vaccine strain of *L. major* originally isolated from a patient in Iran kindly provided by R. Behin, WHO Immunology Research and Training Centre, Lausanne, Switzerland and a Kenyan strain of *L. donovani* (MHOM(/KE/85/NLB 274) kindly provided by Kenya Medical Research Institute, Nairobi, Kenya. Promastigotes were cultured in medium 199 containing 0.02 mg/ml gentamycin, 25 mM Hepes, 4 mM L-glutamine, and 20% heat inactivated fetal calf serum (56°C, 30 min). Incubation was carried out at 26°C. Promastigotes were harvested on day 3 and 6 of the culture and used for the parasiticidal assay.

Drugs. Licochalcone A was purified from Chinese licorice roots as described in Example 1. 1 mg of licochalcone A was dissolved in 20 µl of 99% (v/v) ethanol, and then added to 980 µl of medium 199, stored at -20°C.

Effect on promastigotes. The effect of licochalcone A on promastigotes was assessed by a method similar to the one described by Pearson et al., by incubating promastigotes ($3x10^6$/ml) at 26°C for 2 hrs in the presence of licochalcone A or the medium alone in 96 wells flat bottom microtiter plates. Following incubation, 100 µCi of [^{3}H] thymidine was added to each well and further incubated for 18 hrs. Promastigotes were then harvested on filter paper by means of a cell harvester (Skatron, Lierbyen, Norway), extensively washed with distilled water and counted in a scintillation counter (Minaxi Ti-Carb 4000, United Technologies, Packard, USA). The promastigotes were also counted microscopically and their flagellar motility was assessed.

Results

The antileishmanial activity of licochalcone A was tested on promastigotes of *L. major* and *L. donovani* at both logarithmic and stationary stages of growth. The 3-day culture was taken as the logarithmic and the 6-day culture as the stationary stage promastigotes. Licochalcone A inhibited the growth of promastigotes of both *L. major* and *L. donovani* in a concentration-dependent manner (Table 4.1). A significant reduction of growth of promastigotes of *L. donovani* at 3-day and 6-day cultures was observed at 5 µg/ml. The licochalcone A exhibited a stronger inhibitory effect on the 6-day culture as compared to the 3-day culture of the parasite. The 50% inhibition of the log phase promastigotes was reached at a concentration of licochalcone A between

2 μg/ml and 5 μg/ml whereas the 50% inhibition of the stationary phase promastigotes was reached at a concentration of licochalcone A around 2 μg/ml. At 20 μg/ml of Licochalcone A there was total inhibition of promastigotes growth at both stages. More than 90% inhibition of promastigote growth was observed with 10 μg/ml on the growth of *L. major* 3-day cultures and 6-day cultures and the growth of *L. donovani* 6-days cultures.

The stationary phase (6-day culture) which is known to be the infective form of the parasite was more sensitive than the log phase (3-day culture).

Table 4.1. Comparison of the effect of licochalcone A on *L. major* and *L. donovani* promastigotes from 3-days and 6-days cultures. The results are from 5 experiments and are given as mean ± SEM percentage inhibition of ^{3}H-thymidine uptake in control promastigotes grown in medium alone.

Licochalcone A (μg/ml)	*L. major* 3-day	*L. major* 6-day	*L. donovani* 3-day	*L. donovani* 6-day
20	96.0±7.5	99.4±1.1	96.7±0.8	98.3±0.8
10	91.9±8.4	96.4±1.2	81.5±8.0	91.9±3.1
5	63.9±13.0	80.7±6.4	53.7±14.0	70.1±12.4
2	14.8±21.5	42.6±19.7	23.5±16.4	42.2±16.6
1	3.6±11.5	15.8±16.5	15.2±14.5	30.4±23.8

Conclusion

The major importance of this finding was that licochalcone A at non-toxic concentrations inhibited the growth of the extracellular promastigote stage of both *L. major* and *L. donovani*. The lethal effect of licochalcone A on *L. donovani*, the causative agent of the fatal visceral leishmaniasis, is important especially in the light of resistance development against antimonials, the only antileishmanial drugs in use.

Licochalcone A was lethal to both the infective and the non-infective promastigote forms of the parasite, and therefore has the potential of preventing macrophage infection in a prophylactic manner.

EXAMPLE 5

Effect of seven bis-aromatic α,β-unsaturated ketones on the *in vitro* growth of *L. major* promastigotes

Materials and methods

Parasite cultures. The same parasite culture as described in Example 4. Promastigotes were harvested on day 4 of the culture and used for the parasiticidal assay.

Drugs. Seven bis-aromatic α,β-unsaturated ketones prepared as described in Example 2.

Effect on promastigotes. The effect of seven α,β-unsaturated bis-aromatic ketones was assessed by a method similar to the one described by Pearson et al. (1984) by incubating promastigotes ($3x10^6$/ml) at 26°C for 2 hrs in the presence of drugs or the medium alone in 96 wells flat bottom microtiter plates. Following incubation, 100 μC of ^{3}H-thymidine was added to each well and further incubated for 18 hrs. Promastigotes were then harvested on filter paper by means of a cell harvester (Skatron, Lierbyen, Norway), extensively washed with distilled water and counted in a scintillation counter (Minaxi Ti-Carb 4000, United Technologies, Packard, USA).

Results

Table 5.1. Comparison of the effect of seven bis-aromatic α,β-unsaturated ketones on *L. major* promastigotes from 4-days cultures. The results are given as mean±SEM percentage inhibition of ^{3}H-thymidine uptake in control promastigotes grown in medium alone.

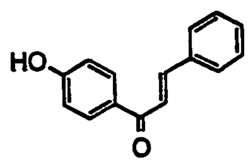
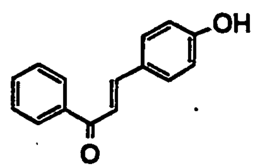
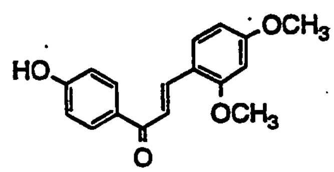
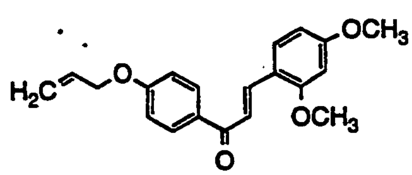
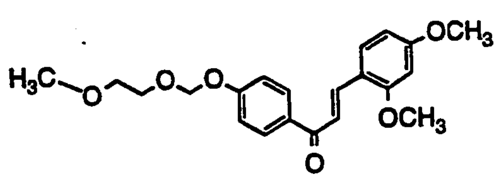
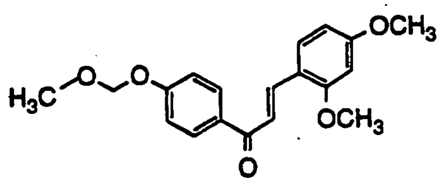
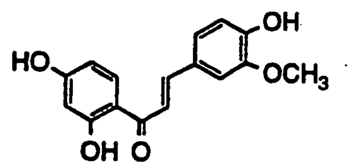
drug (μg/ml)		n	mean ± SEM
20		7	91.9±2.1
10		7	82.8±4.1
5		7	70.8±4.6
1		7	35.9±3.2
20		7	94.3±1.6
10		7	84.5±2.8
5		7	70.1±5.0
1		7	39.1±7.8
10		3	91.9±4.6
5		3	61.8±6.8
1		3	27.3±7.6
0.5		3	19.4±5.6
10		3	84.3±3.5
5		3	64.5±4.6
1		3	17.5±5.8
0.5		3	7.5±6.9
10		3	60.5±5.6
5		3	31.0±6.7
1		3	14.0±4.5
0.5		3	20.0±7.5

Table 5.1 continued

		n	mean±SEM
10		2	65.0±5.9
5		2	56.3±7.6
1		2	18.1±7.8
0.5		2	8.5±5.6
10		2	83.3±4.6
5		2	66.7±5.5
1		2	14.3±6.4
0.5		2	7.8±6.7

EXAMPLE 6

Effect of licochalcone A on the *in vitro* growth of *L. major* and *L. donovani* amastigotes

Materials and Methods

Macrophage culture. The method used in this study was a modification of the one described by Berman et al. (1979). Human peripheral blood monocytes were obtained by Ficoll-Hypaque fractionation of blood from the Blood Bank, Rigshospitalet, Copenhagen, Denmark. Washed cells were suspended in medium RPMI-199 containing penicillin (50 U/ml), and streptomycin (50 µg/ml), 25 mM Hepes, 4 mM L-glutamine, and 10% heat inactivated fetal calf serum (56°C, 30 min). One milliliter of suspension containing $1x10^6$ mononuclear cells was added to each well (16 mm in diameter, with one piece of cover glass in 12 mm diameter) of 24 wells of plastic culture trays (Nunc, Denmark). After 4 h and again after 3 days of incubation at 37°C in 5% CO_2-95% air, old medium was removed and washed with warm fresh medium three times, and then replaced with warm fresh medium.

Infection of macrophages with *L. major* promastigotes. After 6 days incubation the old medium was removed, and one milliliter of $1x10^7$ 4 days culture of *L. major* promastigotes (same promastigotes culture as in Example 4) was added to each well. After 24 h infection, macrophage cultures were washed three times with warm fresh medium and replenished with warm fresh medium with different concentrations of licochalcone A or medium alone. The medium was changed every 3 days. After infection, day 3 and day 6 a certain number of macrophage cultures were fixed with absolute methan-

ol, stained with 5% Giemsa stain for 10 min, and examined by light microscopy (x1,000). The percentage of macrophages that contained amastigotes and the number of amastigotes present per infected macrophage were determined in replicate cultures by counting 200 cells per well.

Results

As shown in Table 6.1 the number of amastigotes/infected macrophage/100 macrophages was reduced from 299/63/100 in controls to 11/5/100 in the presence of 5 µg/ml of licochalcone A.

Table 6.1. Effect of licochalcone A on *L. major* amastigotes. Human macrophages were infected with promastigotes. After 24 hrs the free promastigotes were removed. The infected macrophages were then incubated with licochalcone A for 3 days and 6 days. The results are presented as the number of amastigotes/number of infected macrophages/100 macrophages.

Licochalcone A (µg/ml)	0-day	3-day	6-day
10	237/51/100	10/5/100	6/4/100
5	"	18/7/100	11/5/100
1	"	54/43/100	45/45/100
0	"	252/52/100	299/63/100

Conclusion

The amastigote phase of the parasite is the phase to which the parasite converts to in the macrophages of the host. From both tables it is seen that not only the total amount of parasites was reduced, but also the amount of cells infected was reduced when treated with licochalcone A.

EXAMPLE 7

Effect of licochalcone A on the *in vitro* multiplication of *Leishmania major* amastigotes in human macrophages and U937 cells

Materials and methods:

Drugs. Licochalcone A was purified from Chinese licorice roots as described in Example 1.

Human PBM-derived (Peripheral Blood Mononuclear cells) macrophage culture. The methods which were used in this study are a modification of the methods described by Berman et al. Human peripheral blood monocytes, obtained by Ficoll-Hypaque fractionation, and washed cells were suspended in medium RPMI-199 containing 550 U/ml of penicillin and 50 U/ml of streptomycin, 25 mM Hepes, 4 mM L-glutamine, and 10% heat inactivated fetal calf serum at 56°C for 30 min. One ml of suspension containing $5x10^6$ mononuclear cells was added to each well (16 mm in diameter, with one piece of cover glasses in 12 mm diameter) of 24 wells of plastic culture trays (Nunc, Denmark). 200 µl of $5x10^6$ mononuclear cells were added to each well in flat-bottom microtiter plates (Nunc, Denmark). After 4 h and again after 3 days of incubation at 37°C in 5% CO_2-95% air, old medium was removed and washed with warm fresh medium three times and then replaced with warm fresh medium.

U937 cell culture. U937 cells were maintained at 37°C as suspension cultures in the same medium as the one used for macrophages culture. Two days before infection, U937 cells were treated with 10 ng/ml of phorbol myristate acetate (PMA, L.C. Services, Woburn, Mass.) in the culture medium. This caused the cells to differentiate into a non-dividing adherent monolayer.

Promastigotes culture. The *L. major* (WHO vaccine strain) promastigotes were obtained from footpad tissue of BALB/c mice infected 1 to 2 months previously by subcutaneous inoculation with $1x10^7$ stationary phase promastigotes. The promastigotes were passaged in culture two times before use. The culture was the same as the one used in Example 6.

Infection of macrophages with *L. major* promastigotes. After 6 days incubation the old medium was removed, and one ml or 200 µl of $1x10^7$/ml 6 days culture of *L. major* promastigotes as described in Example 6 was added to each well, and the cultures were carried out at 34°C. After 24 h infection, macrophages were washed three times with warm fresh medium and replenished with warm fresh medium with different concentrations of licochalcone A or medium alone.

Infection of U937 cells with *L. major* promastigotes. Differentiated U937 cells were infected with *L. major* promastigotes as described above for macrophages.

<u>Measurement of intracellular parasite killing</u>

Killing was quantified by a modification of a technique described by Berman et al.

Briefly, 3 days after infection, microtiter well cultures were rinsed once with medium, exposed to 100 µl of prewarmed (37°C) 0.01% sodium dodecyl-sulfate (SDS) in medium RPMI 1640, and returned to 34°C for 15 min. By this procedure, macrophages were lysed, according to microscopic observation, and amastigotes were released. 100 µl of medium RPMI 1640 with 20% HFCS were added to each well without removal of the lysing solution. The plates were then transferred to a 26°C incubator, and amastigotes were left to transform to promastigotes. After 48 h, parasite growth was recorded by adding 1 µCi of ^{3}H-thymidine (New England Nuclear Corp., Boston, Mass.) to each well. The parasites were harvested 24 h later on glass fiber filters with a harvesting machine (Skatron, Lierbyen, Norway), and ^{3}H-thymidine incorporation was measured in a liquid scintillation counter (Tricarb; Packard Instrument Co., Inc., Rockville, Md.). The parasite survival index (PSI) was determined. This index is the mean ^{3}H-thymidine incorporation (cpm) in treated infected cells as compared with the untreated infected cells.

$$PSI = \frac{\text{cpm of treated infected cells}}{\text{cpm of untreated infected cells}} \times 100$$

3 days and 6 days after infection, macrophage cultures on cover glasses were fixed with absolute methanol, stained with 5% Giemsa stain for 10 min and examined by light microscopy (x1,000). The percentage of macrophages that contained amastigotes and the number of amastigotes and promastigotes present per infected macrophage were determined in replicate by counting 200 cells per well in microscope.

Results

The PSI-index was plotted against the concentration of licochalcone A. The plots are shown in Fig. 1 and Fig. 2.

Table 7.1. Effect of licochalcone A on *L. major* amastigotes in U937 cells (macrophage cell line). The data is given as mean percentage of 3 experiments. Microscopic counting.

Licoch. A (µg/ml)	% of infec. cells	amast. per cell	proma. per cell	% of amast.	total amast. ($x 10^6$)	total proma. ($x 10^6$)
0 days after infection						
	40.7	2.9	0.4	86.9	117	17.7
3 days after infection						
10	0					
5	0					
1	19.0	2.0	0.2	91.2	38.3	3.7
0.5	28.0	2.3	0.1	96.6	65.0	2.3
control	43.3	5.7	0.2	97.2	258.3	7.0
6 days after infection						
10	0					
5	0					
1	3.0	1.8	0	100	5.3	0
0.5	13.7	3.1	0	100	42.0	0
control	47.3	6.3	0.02	99.9	298.3	2.0

Table 7.2. Effect of licochalcone A on *L. major* in human PBM-derived macrophages. The data is given as mean percentage of 3 experiments. Microscopic counting.

Licoch. A (μg/ml)	% of infec. cells	amast. per cell	proma. per cell	% of amast.	total amast. ($\times 10^6$)	total proma. ($\times 10^6$)
0 days after infection						
	36.3	2.1	0.2	91.4	76.7	7.3
3 days after infection						
10	0					
5	0					
1	10.7	1.7	0.2	88.2	17.7	2.0
0.5	14.0	1.7	0.1	91.6	23.3	2.0
control	44.0	3.6	0.1	98.0	157.7	3.3
6 days after infection						
10	0					
5	0					
1	7.3	2.2	0	100	16	0
0.5	8.0	1.7	0	100	14	0
control	44.0	4.4	0.1	100	99.6	0

Discussion

The major importance of this finding is that licochalcone A at non-toxic concentrations inhibited the growth of both the extracellular promastigote stage and the intracellular amastigote stage of *L. major*. By comparing the results shown in Table 7.1 and Table 7.2 with the results shown in Fig. 1 and Fig. 2 it is seen, e.g. for infected U937 cells treated with licochalcone A in a concentration of 1 μg/ml, that although 2.0 amastigotes per cell were seen in the microscope in the treated group compared to 5.7 amastigotes in the control group, the PSI index for the same concentration of licochalcone A was only about 5%. This means that many of the parasites seen in the

microscope were killed and therefore not able to take up ^{3}H-thymidine. Therefore, the best method to measure the intracellular killing of the parasites is the PSI-index. Inhibition of growth and multiplication of the amastigote form of the parasite is crucial, since *Leishmania* parasites exist solely inside macrophages during established infection.

EXAMPLE 8

Effect of licochalcone A on the *in vivo* growth of *L. major*

Materials and Methods

Mice. BALB/c female mice aged eight weeks old were used throughout.

Parasite. The maintenance, cultivation, and isolation of the promastigote-stage of the parasite *L. major* (WHO reference vaccine strain) have been described in detail in Example 4. For animal infection, 6 groups of 10 mice each received s.c. injections (in 0.05 ml of PBS) in the left hind footpad with $1x10^7$ stationary phase promastigotes.

The lesions that developed in the footpad were measured with a dial-calliper and expressed as footpad thickness increase (in mm). The footpad thickness of mice was measured before infection and every 3 days after 7 days of infection. From 7 days of infection, mice received licochalcone A injections i.p. or intralesionally once a day. After 42 days of licochalcone A injection, some of the mice were killed and the footpads, spleens and livers removed. The parasite loads in the footpads and livers were estimated by a modification of the method described by Liew et al. 1990. Briefly, the tissues were cut and minced into very small pieces, and supernatants containing the released parasites were cultured in 15 ml of medium RPMI 199 containing 0.02 mg/ml gentamycin, 25 mM Hepes, 4 mM L-glutamine, and 20% heat inactivated fetal calf serum (56°C, 30 min) in 25 cm^2, 50 ml culture flask (Nunc, Roskilde, Denmark). Incubation was carried out at 28°C for 3 days and then pulsed with 1 μCi of ^{3}H-thymidine. Cultures were harvested 18 h later on filter paper by a cell harvester (Skatron, Lierbyen, Norway), extensively washed with distilled water and counted in a scintillation counter (Minaxi Ti-Carb 4000, United Technologies, Packard, USA). The results were expressed as cpm. The footpads, spleens and livers impression was also estimated.

Drugs. One mg of licochalcone A was dissolved in 20 μl of 99% (v/v) ethanol, and then 980 μl of medium 199 was added an the resulting mixture was stored at -20°C before use.

Results

Table 8.1. Effect of licochalcone A on the parasitic load of the footpad of the mice infected with *L. major*. The results are from 2 mice from each group and are given as mean x10^3 cpm of ^{3}H-thymidine uptake.

Group	mean
1) 100 µg i.p.	89.0
2) 50 µg i.p.	58.0
3) 50 µg intralesional	282.6
4) 20 µg intralesional	243.5
5) Buffer i.p.	357.6
6) Buffer intralesional	485.4

Table 8.2. Effect of licochalcone A on the parasitic load of the liver of the mice infected with *L. major*. The results are from 2 experiments and are given as mean x10^3 cpm of ^{3}H-thymidine uptake.

Group	mean
1) 100 µg i.p.	12.8
2) 50 µg i.p.	11.7
3) 50 µg intralesional	20.8
4) 20 µg intralesional	10.8
5) Buffer i.p.	184.0
6) Buffer intralesional	90.8

Table 8.3. Effect of licochalcone A on *L. major* parasite in the footpad, spleen and liver of the mice infected with *L. major*. The results are given as amastigotes findings on the impression smear.

Group	Footpad	Spleen	Liver
1) 100 µg i.p.	+	-	-
2) 50 µg i.p.	+	-	-
3) 50 µg intralesional	+	+	+
4) 20 µg intralesional	+	+	+
5) Buffer i.p.	+++	++	++
6) Buffer intralesional	+++	++	++

- no parasite amastigotes detected in 5 fields of microscopical vision
+: parasite amastigotes from 5 fields of microscopical vision < 100
++: parasite amastigotes from 5 fields of microscopical vision=100-500
+++: parasite amastigotes from 5 fields of microscopical vision=500-1000

Fig. 3 shows the effect of licochalcone A on footpad thickness increase (swelling) in BALB/c mice infected with *L. major*, expressed in mm.

<u>Conclusion</u>

It appears that intraperitoneal administration of licochalcone A gives a better effect than intralesional administration. This could be due to leaking of the administered drug from the lesions. However, the latter administration form is also effective compared to the results from the group treated with buffer.

From Fig. 3 the same picture is seen, in that the increase in the footpad thickness is less in the group treated intraperitoneally compared to the group treated intralesionally. The footpad thickness is an expression of the degree of disease, in that the more the infected footpad is swollen the more disease has developed.

These data clearly demonstrate that both intraperitoneal and intralesional administration of licochalcone A prevents lesion development in mice caused by *Leishmania* infection.

EXAMPLE 9

Effect of licochalcone A on *L. donovani* infection in hamsters

Animals. Male Syrian golden hamsters (Mesocricetus auratus), 50-70 g body weight, were used throughout.

Parasite. *L. donovani* (MHOM/KE/85/NLB 439) promastigotes were cultured in medium 199 containing 0.02 mg/ml gentamycin, 25 mM Hepes, 4 mM L-glutamine, and 10% heat inactivated fetal calf serum (56°C, 30 min).

Drugs. Licochalcone A was dissolved in 20 µl of 99% (v/v) ethanol, and the 980 µl of medium 199 was added, and the resulting mixture was stored at -20°C.

Animals were intracardially inoculated with 2 x 10^7 *L. donovani* promastigotes in 0.1 ml medium 199 (Day 0). One hour later, one of the animals was killed. The liver and the spleen were weighed. The liver and the spleen impression smears were made. After air-drying, the impression smears were fixed with water-free methanol and stained with Giemsa. Five of the animals were treated (i.p.) with licochalcone A (10 mg/kg body weight two times per day) from Day+1 to Day+7. Another five animals were treated with 0.85% NaCl. The animals were killed on Day+8. The liver and spleen were weighed, and the liver and the spleen impression smears were made. The number of the parasite in the liver and the spleen were counted under microscope. The spleen of the animals were cut into very small pieces, cultured in 15 ml of the culture medium at 26°C overnight. The spleen cultures were centrifuged at 1,000 rpm for 10 min and then, the supernatant were removed and the residue was recultured in the same medium for five days at 26°C. 200 µl of the spleen culture was added into one well of 96 wells flat bottom microtiter plates (triplicate). 100 µC of ^{3}H-thymidine were added to each well and the incubation was continued for 18 h. Promastigotes were then harvested on filter pater by means of a cell harvester (Skatron, Lierbyen, Norway), extensively washed with distilled water and counted in a scintillation counter (Minaxi Ti-Carb 4000, United Technologies, Packard, USA).

Results and conclusions

As shown in Fig. 8, the parasite load both in the liver and the spleen of animals receiving intraperitoneal injections of 10 mg per kg body weight licochalcone A two times per day for 7 days was reduced to almost undetectable levels.

The *in vivo* inhibitory effect of licochalcone A on *L. donovani*, the causative agent of the fatal visceral leishmaniasis, is quite promising especially in the light of resistance

development against antimonials, the only antileishmanial drug in use.

EXAMPLE 10

Effect of Licochalcone A on pentostam resistant *L. major* promastigotes

The experiment was performed the same way as described in Example 4.

Results and conclusions

The results are shown in Figures 9A, 9B and 9C. As it can be seen from the figures, licochalcone A at concentrations of 5 µg/ml (Figure 9A) completely inhibited the growth of pentostam resistant *Leishmania* parasites. Combination of licochalcone A with 10 µg/ml pentostam resulted in an increased inhibitory effect by licochalcone A on pentostam resistant *Leishmania* parasites (Figure 9C).

EXAMPLE 11

Effect of different concentrations of licochalcone A on the ultrastructure of *Leishmania major*

Electron microscopic studies. In order to examine the effect of licochalcone A on the ultrastructure of the parasite, electron microscopy studies were carried out on promastigotes and amastigotes incubated with different concentrations of licochalcone A.

Materials and Methods

Drugs. Licochalcone A was dissolved in 20 µl of 99% (v/v) ethanol, and then 980 µl of medium 199 was added and stored at -20°C.

Effect on promastigotes. The effect of licochalcone A on promastigotes was assessed by incubating promastigotes ($3x10^6$/ml, 5 ml) at 26°C for 20 hrs in the presence of licochalcone A or the medium alone in plastic tubes. Cultures were centrifuged at 1.500 rpm for 10 min, supernatants were removed, and the pellets were resuspended in 2 ml of 3% glutaraldehyde in 0.1 M cacodylate buffer pH 7.3.

Macrophage culture. Human peripheral blood monocytes were obtained by Ficoll-Hypaque fractionation of blood from the Blood Bank, Rigshospitalet. Washed cells were suspended in medium RPMI-199 containing penicillin (50 U/ml), and streptomycin (50 µg/ml), 25 mM Hepes, 4 mM L-glutamine, and 10% heat inactivated fetal calf serum (56°C, 30 min). 15 ml of suspension containing $1x10^6$ mononuclear cells per ml

was added to each flask (25 cm^2, 50 ml, Nunc, Roskilde, Denmark). After 4 h and again after 3 days of incubation at 37°C in 5% CO_2-95% air, old medium was removed and washed with warm fresh medium three times, and then replaced with warm fresh medium.

Infection of macrophages with *L. major* promastigotes. After 6 days incubation the old medium was removed, and 15 ml of $1x10^7$/ml 4 days culture of *L. major* promastigotes was added to each flask. After 24 h infection, macrophage cultures were washed three times with warm fresh medium and replenished with warm fresh medium with different concentrations of licochalcone A or medium alone. The medium was changed every 3 days. After 6 days, the medium was removed and 4 ml of a suspension of Versene (EDTA) and Trypsin (prepared by adding 3 ml of 0.2% Trypsin to 50 ml of Versene) was added, and shaken a few times. After 5-10 min (the adherent cell layer should loosen in 5-10 min), 4 ml of 6% glutaraldehyde in 0.1 M cacodylate buffer pH 7.3 was added to the flasks and the cells were fixed for 20 min at room temperature.

Promastigotes of *L. major* grown in media for 24 hours at 26°C with different concentrations of licochalcone A. The number of organisms was 3 x 10^6/ml.

Sample A: *L. major* grown in media alone (control)
Sample B: *L. major* grown in media with 1 µg/ml licochalcone A
Sample C: *L. major* grown in media with 5 µg/ml licochalcone A
Sample D: *L. major* grown in media with 10 µg/ml

After 24 hours of incubation, 5 ml of each sample was centrifuged (1500 rpm), and the pellets were resuspended in 1 ml of media. Then the organisms were fixed by adding 1 ml of 6% glutaraldehyde in 0.1 M cacodylate buffer containing 0.01 M CaCl pH 7.3.

After 2 hours of fixation at room temperature, the specimens were centrifuged (Eppendorf 8000 rpm) for 2 min, and the pellets were enrobed in 45°C melted 1.5% Noble Agar (Difko) in 0.1 M cacodylate buffer containing 0.01 M $CaCl_2$ pH 7.3, followed by en block staining in 2% uranylacetate in barbiturate buffer pH 7.2 for another hour.

The agar blocks with cells were then dehydrated in alcohol and propylene oxide and finally embedded in Vestopal-W. After hardening of the blocks, sections were obtained on the LKB-ultratome III microtome. The sections were post-stained for 15 min with magnesium uranyl acetate and afterwards for 2 min with lead citrate diluted 1:10 with redistilled water.

Electron microscopy was carried out with a Philips EM 201 C electron microscope. Exposures were made on Eastman Kodak Fine Grain Release Positive Film Type 5302 at primary magnifications of 1500 and 9000 x and suitable fields were enlarged photographically ten times.

Results

The ultrastructure of *L. major* in the control sample showed a uniform population of cells, all with flagella and rounded uncondensed nuclei, see Fig. 5.

The cytoplasm contained very few profiles of rough endoplasmic reticulum, whereas well developed golgi complexes were found in several cells together with electron-dense and electron-lucent granules.

The kinetoplast and its associated mitochondrion were slender and contained a few cristae. Long slender mitochondria were also found surrounding the nuclei and the golgi complex.

After 1 µg/ml of licochalcone A no morphological changes could be found, compared to the control culture.

After 5 µg/ml of licochalcone A a higher percentage of *L. major* showed cytoplasmic granules than the control culture (70% versus 50%). However, the most spectacular changes were seen in the ultrastructure of the mitochondria. These were swollen to an extent that made it difficult to recognize the structures as mitochondria, if the characteristic cristae had not been preserved.

10 µg/ml of licochalcone A increased the above mentioned ultrastructural change (see Fig. 6).

Table 11.1 Shows the measurements of the diameters of mitochondria, measured on prints at a magnification of 15000 x.

	n	Maximum (mean) (nm)	n	Minimum (mean) (nm)
A Control	20	170	4	70
B 1 µg/ml	10	203	4	104
C 5 µg/ml	28	548	7	165
D 10 µg/ml	20	1043	5	294

EXAMPLE 12

Effect of different concentrations of licochalcone A on the ultrastructure of human monocytes/macrophages with and without ingested *L. major* with special reference to the ultrastructure of the mitochondria in *L. major* and in the human mononuclear cells

<u>Materials and methods:</u>

Human mononuclear cells isolated from the same donor were cultivated in 200 ml Nunc flasks, each containing 30 ml of $5x10^6$/ml cells. They were grown for 6 days, whereby the macrophage cells will adhere to the bottom. Media were changed after 3 days. After 6 days the *L. major* promastigotes were added in a concentration of 1×10^7 to four of the specimens, and incubated together for 24 hours. Then the media were exchanged with media containing Licochalcone A in different concentrations. The cultures were then grown for 6 days.

After the media were sucked off, the adhering cells were loosened by adding 8 ml of a mixture of EDTA and Trypsin (3 ml of 0.2% trypsin to 50 ml of EDTA). After 5 min the cells loosened, and the cells were fixed by adding 4 ml of 6% glutaraldehyde in cacodylate buffer. After 20 min of fixation, the cells were transferred to polypropylene tubes, and the further preparation and treatment took place as described in Example 10.

<u>Results</u>

A) Macrophage + *L. major* + 0 µg/ml licochalcone A Control culture. Cells with up to 20 amastigotes were seen. The ultrastructure of the mitochondria was well preserved

in both the human cells and in *L. major*.

B) Macrophage + *L. major* + 1 μg/ml licochalcone A. The ultrastructure of the cells in this experiment did not deviate from A.

C) Macrophage + *L. major* + 5 μg/ml licochalcone A.The amastigotes found in the cytoplasm of the mononuclear cells showed distended mitochondria, whereas no ultrastructural changes could be seen in mitochondria of the human mononuclear cells.

D) Macrophage + *L. major* + 10 μg/ml licochalcone A. Most of the intracellular amastigotes were now killed by licochalcone A and degraded by the macrophages, so it was often difficult to recognize the structure of an amastigote. Some were however found with the characteristic distension of the mitochondria. Again no ultrastructural changes were seen in the mitochondria of the macrophage.

E) Macrophage grown alone control culture. Well preserved ultrastructure and mitochondria.

F) Macrophage + 5 μg/ml licochalcone A. No changes compared to the control culture.

G) Macrophage + 10 μg/ml licochalcone A. No changes compared to the control culture (see Fig. 11).

Conclusion

These results show that the mitochondria and the other organelles of the cell are not in any way deteriorated by the licochalcone A in concentrations necessary to kill the parasites.

EXAMPLE 13

Effect of licochalcone A on the function of human lymphocytes, polymorphonuclear leucocytes, and monocytes

Materials and methods

Lymphocyte proliferation. Human blood mononuclear cells (BMNC) from heparinized blood were isolated by metrizoate sodium-Ficoll (Lymphoprep, Nyegaard, Oslo, Norway) density gradient centrifugation, washed 3 times in RPMI 1640 medium

(Gibco) supplemented with 5% fetal calf serum (FCS) and with 400 IU of penicillin plus 400 µg/ml streptomycin.

BMNC were resuspended in the medium and cultured in triplicate, 0.63x10^5/ml and 160 µl per vial, in round-bottom microtiter plates (Nunc, Roskilde, Denmark) with 20 µl of various concentrations of licochalcone A. Immediately prior to incubation, optimum concentrations of the mitogen phytohaemagglutinin (PHA) and the antigen purified protein derivative of tuberculin (PPD) were added to the cultures in a volume of 20 µl. Unstimulated control cultures were always included.

Cultures were incubated for 3 or 7 days. The degree of lymphocyte proliferation was estimated by ^{3}H-thymidine (1 µCi per well; New England Nuclear Corp., Boston, Mass.) addition 24 h before the cells were harvested on glass fiber filters by means of a harvesting machine (Skatron, Lierbyen, Norway), and ^{3}H-thymidine incorporation was measured in a liquid scintillation counter (Tricarb; Packard Instrument Co., Inc., Rockville, Md.). For each set of triplicate values, the median was recorded. Unstimulated cultures were always included as controls.

Chemiluminescence. Monocytes were prepared from heparinized blood by metrizoate sodium-Ficoll (the same as lymphocyte proliferation). Polymorphonuclear leucocytes (PMN) were prepared from heparinized blood by dextran sedimentation and metrizoate sodium-Ficoll separation. Remaining erythrocytes were removed by hypotonic lysis. A zymosan-enhanced chemiluminescence assay was used. The assay was performed in a total volume of 5.5 ml at ambient temperature in glass scintillation vials. A Beckman L 8000 scintillation counter (placed under air-conditioned, thermostat controlled, 21±1°C conditions) was used in the out-of-coincidence mode. A 1.1 ml portion of PMN or monocyte suspension (1x10^6 cells per ml) was pre-incubated with 1.1 ml of various concentrations of licochalcone A for 30 min at 37°C in a rotor at 20 rpm. Luminol (5-amino-2,3-dihydro-1,4-phatalazinedione) obtained from Sigma Chemical Co., St. Louis, Missouri, U.S.A., was maintained as a stock solution of 10 mg/ml in NaOH (0.1 N) and diluted in Krebs-Ringer solution immediately before use. Each vial contained 5x10^5 pre-incubated PMN or monocyte, 4 mg of opsonized zymosan (Sigma), 50 µl of luminol solution, and 4.45 ml of Krebs-Ringer solution containing 5 mM glucose. The final concentration of luminol in the assay mixture was 5x10^{-8}M.

Trypan blue dye exclusion method. This is a standard method used to determine cell viability. The assay is performed by incubating a given cell suspension with trypan blue for various periods of time after which the cells are examined under microscope. Dead cells will take up the dye and therefore show a blue colour. Percent viability is determined by counting at least a total of 200 cells.

Drug. Licochalcone A was purified from a batch Chinese licorice roots as described in Example 1. 1 mg of licochalcone A was dissolved in 20 µl of 99% (v/v) ethanol, and then added in 980 µl of medium 199, stored at -20°C.

Results

Licochalcone A in a concentration of 20 µg/ml had no toxic effect on human lymphocytes, neutrophils, and monocytes as measured by the trypan blue dye exclusion method.

Table 13.1 shows that licochalcone A decreased the human lymphocyte proliferation response to PHA and PPD in concentrations of 20 µg to 10 µg/ml. Table 13.2 shows that licochalcone A caused a marked decrease in chemiluminescence response of human PMN and monocyte to opsonized zymosan in concentrations of licochalcone A of 20 µg/ml to 10 µg/ml.

Table 13.1. Effect of licochalcone A on human lymphocyte proliferation response to PHA and PPD as measured by ^{3}H-thymidine incorporation. Results are given as percentage inhibition of control cells response ±SEM in the absence of licochalcone A (7 experiments).

Licochalcone A (µg/ml)	PHA	PPD
20	65.3±7.0 *	64.4±12.0 *
10	39.7±5.4 *	58.3±14.2 *
5	20.8±4.8 *	16.3±7.3
2	7.8±5.7	7.2±7.2
1	4.7±5.6	11.3±5.3

* P<0.05.

Table 13.2. Effect of licochalcone A on chemiluminescence response of human PMN and monocytes to opsonized zymosan. Results are given as mean±SEM percentage inhibition of control cells response in the absence of licochalcone A (7 experiments).

Licochalcone A (µg/ml)	PMN	Monocytes
20	47.0±4.3 *	56.5±3.8 *
10	29.7±4.8 *	37.9±3.1 *
5	18.9±3.7 *	24.8±5.3 *
2	10.3±3.3 *	7.8±6.5
1	2.1±3.3	8.2±3.3

* $P<0.05$.

EXAMPLE 14

Effect of a number of bis-aromatic α,β-unsaturated ketones on *L. major* promastigotes from 4-days cultures on *Plasmodium falciparum* growth *in vitro* and on human lymphocyte proliferation response to PHA

Materials and methods

Drugs. Licochalcone A and a large number of analogues, some prodrugs, and a few compounds with modified chalcone structure.

Lymphocytes. Lymphocytes were prepared and analyzed as described in Example 13.

***L. major* promastigotes.** The *L. major* promastigotes were prepared as described in Example 4 and harvested after 4 days. The results are given as mean±SEM percentage inhibition of ^{3}H-thymidine uptake compared to control promastigotes grown in medium alone.

The *P. falciparum* experimental setup was as described in Example 15.

The compounds in Table 14.1 marked with an asterisk are comparison compounds which clearly do not show sufficient potency or selectivity to be useful for the purpose of the invention, whereas the other compounds illustrate the invention.

Table 14.1. The effect of chalcones on *L. major* promastigotes from 4-days cultures, on *P. falciparum* growth *in vitro*, and on human lymphocyte proliferation response to PHA. The upper figures are percentage inhibition of human promastigotes, the middle figures (*italic*) are inhibition of malaria parasites, the lower figures (**bold**) are inhibition of lymphocytes. When standard deviation is given, more than five experiments have been performed.

Hydroxychalcones, and Chalcone

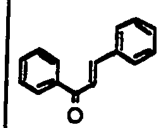
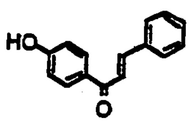
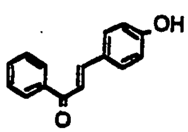
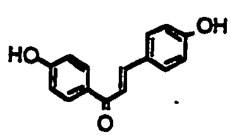
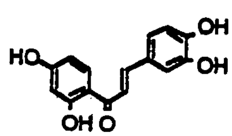
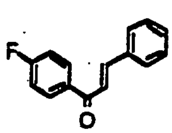
Formula	10 µg/ml	5 µg/ml	1 µg/ml	0.5 µg/ml
* 	97.3 **100**	91.9 **91**	34.8 **7.3**	21.6 **2**
	90.1±6.5 *95.6±1,.* **17.0±18.3**	73.4±15.3 *64.3±8.5* **10.2±13.0**	37.3±13.6 *40.5±11.0* **3.6±6.0**	26.4±6.5 *29.9±10.7*
	91.0±5.4 *88.2±5.3* **14.2±16.7**	80.1±12.6 *61.3±10.6* **7.73±7.1**	49.1±16.8 *29.2±7.7* **2.24±3.3**	7.0 *20.5±11.0*
* 	53.1±7.9 **94.8±5.0**	34.6±10.5 **96.3±4.2**	26.6±7.6 **0±31.7**	22.5±10.0 **0±22.7**
* 	10.6 **51**	4.2 **12**	8 **1**	7 **0**
* 	99	88		

Table 14.1 continued.

Methoxychalcones, and Methoxyhydroxychalcones

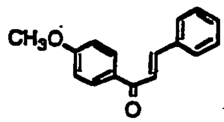
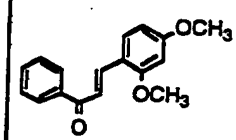
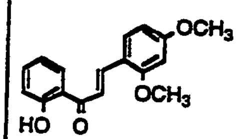
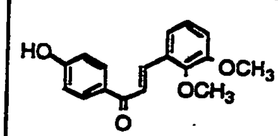
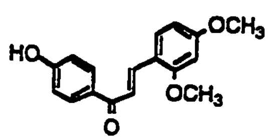
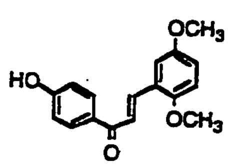
Formula	10 µg/ml	5 µg/ml	1 µg/ml	0.5 µg/ml
* 	85.8 100	68.1 91	26.3 7.5	20.6 2
	95.5±3.8 *81.8* 3.1±15.4	77.8±11.3 *35.6* 2.0±13.2	27.0±13.7 *14.7* 9.2±10.8	14.5±11.6 *0* 9.1±14.0
	98.3±1.0 *67.9* 0±7.0	89.4±13.8 *23.4* 2.6±10.3	37.6±18.3 *0.8* 5.5±12.3	19.2±18.1 *0* 3.0±10.3
* 	97.1±3.5 99.8±0.1	95.2±4.7 89.3±16.2	43.0 7.5±16.5	32.0 2.0±11.0
	90.9±7.4 *64.2* 4.0±15.1	66.9±15.9 *31.6* 2.4±11.1	29.0±22.2 *14.8* 6.3±14.9	20.0±20.6 *2.2* 1.5±11.6
* 	99.1 *93.1* 98.2±2.9	96.2 *71* 78.8±23.0	61.9 *23.1* 6.9±12.7	34.3 *15.2* 3.2±12.5

Table 14.1 continued.

Methoxychalcones, and Methoxyhydroxychalcones

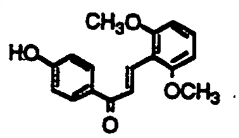
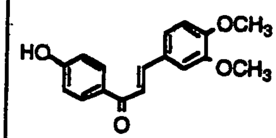
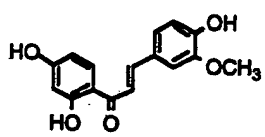
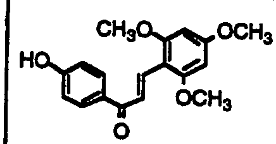
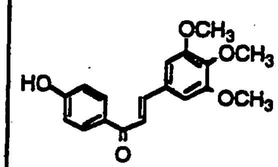
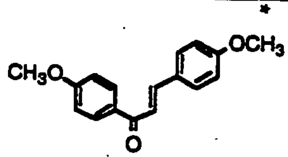
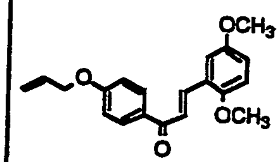
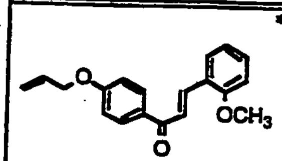
Formula	10 µg/ml	5 µg/ml	1 µg/ml	0.5 µg/ml
	93.1±4.6 *90.6* **4.4±27.9**	77.5±4.3 *71* **2.3±18.4**	21.5±10.6 *16.6* **3.3±12.2**	11.4±9.2 *0* **0±11.5**
	80 *73.4* **27.4±26.8**	65.6 *39.9* **5.2±9.9**	34.3 *35.1* **0.1±8.71**	17.8 *21.0* **0±6.62**
	83.3 **38.3**	56.7 **9.5**	14.3 **6**	7.8 **2**
 *	25.1±17.3 **63.6±15.7**	23.6±10.9 **48±13.3**	25.6±10.9 **0±23.4**	25±9.5 **0±20.5**
 *	24.9±13.1 **1.1±17.2**	26.7±9.6 **0±21.1**	30.5±16.3 **0±19.1**	27.6±8.6 **0±22.7**
 *	51.8±15.8 **0±12.8**	35.6±23.4 **0±22.8**	8.3±4.6 **0±19.2**	11.7±9.2 **0±19.9**

Table 14.1 continued.

Methoxyallyloxychalcones

Formula	10 µg/ml	5 µg/ml	1 µg/ml	0.5 µg/ml
	89.4±10.2 *80* 2.3±12.7	63.0±15.1 *35* 4.3±14.8	15.6±15.1 *0* 12.6±16.6	10.0±9.7 *0* 5.7+13.0
	76.6±22.4 80.1±8.8	52.8±19.1 1.1±27.8	25.8±15.3 0±21.8	20.9±7.7 0±22.5

Methoxyallyloxychalcones

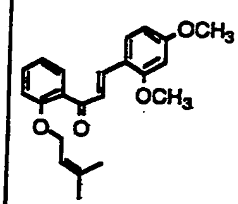
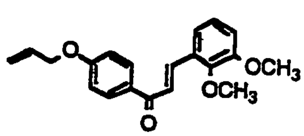
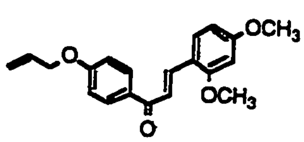
Formula	10 µg/ml	5 µg/ml	1 µg/ml	0.5 µg/ml
	83.2 35.9	49.0 8.7	1.8 6.6	4.3 7.1
	98.1±1.0 *73.1* 13.8±14.6	82.1±13.6 *68.2* 8.8±20.1	20.7±17.1 *17.2* 14.2±20.5	13.9±12.9 *0* 10.8±19.3
	85.4 *97.3* 5.5±18.8	71.4 *74.4* 5.9±9.9	62.8 *36.5* 1.7±11.5	35.4 *31.1* 0±9.8

Table 14.1 continued.

Methoxyallyloxychalcones

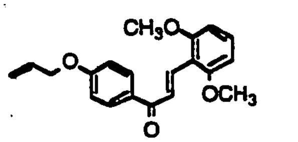
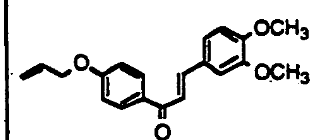
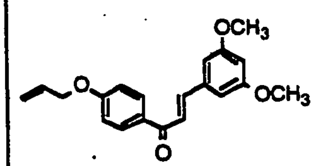
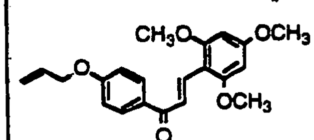
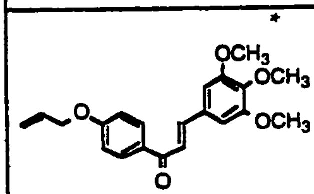
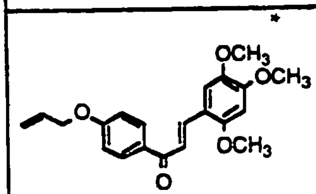
Formula	10 µg/ml	5 µg/ml	1 µg/ml	0.5 µg/ml
	91.1±4.1 *65.7* 0±11.6	63.7±24.2 *39.7* 0±10.2	16.3±12.4 *27.4* 1.3±10.2	11.3±9.8 *0* 1.0±11.4
	90.8±9.7 *68* 15.6±25.0	70.4±15.2 *22.8* 0±8.7	30.4±23.2 *11.4* 0±9.3	15.2±11.3 *2.9* 0±10.6
	98.3±0.9 *98.6* 25.4±16.3	82.8±10.0 *74.3* 4.6±8.4	27.6±23.3 *15.3* 0±7.8	10.6±10.7 *13.8* 0±5.9
* 	22-49 48	14-50 24	15-46 12	25-44 8
* 	91-86 98	44-60 38	16-47 4	11-45 4
* 	28.8±16 27.6±11	23.2±12.8 0±22.7	23.8±12.5 0±26.1	24.0±12 0±23.4

Table 14.1 continued.

Potential Prodrugs

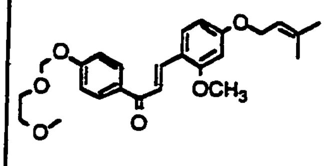
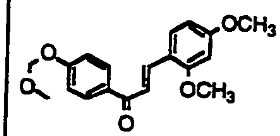
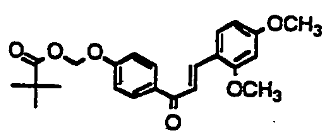
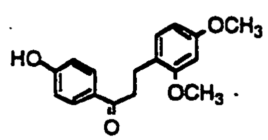
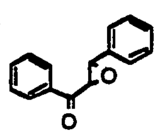
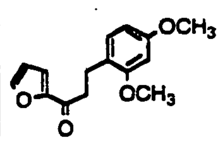
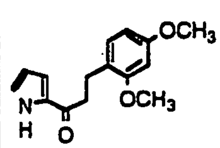
Formula	10 µg/ml	5 µg/ml	1 µg/ml	0.5 µg/ml
	61.5 28.3±11.5	33.1 14.1±7.8	14 8.1±11.2	11.5 0±11.0
	65.4±16.1 15.4±18.7	50.2±13.0 11.2±15.4	27.6±10.9 5.1±11.9	22.8±9.7 0±4.5
	28.6 32.7	7.9 9.0	0 8.2	3.9 7.1

Table 14.1 continued.

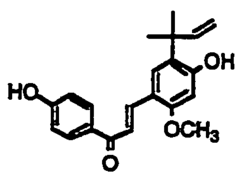
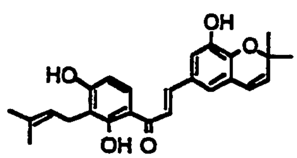
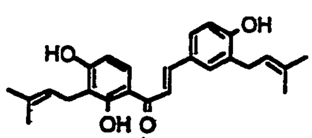
Drugs with a modified chalcone skeleton

Formula	10 µg/ml	5 µg/ml	1 µg/ml	0.5 µg/ml
	31.3±16.0 **1.5±6.2**	22.5±9.7 **3.0±7.0**	5.6±5.4 **0±7.9**	8.5±1.8 **0±8.4**
	88±7.5 **85.4±11**	73.2±17.8 **2±32.2**	33.4±15.4 **0±24.5**	28.4±13 **0±28.3**

Heterocyclic chalcones

Formula	10 µg/ml	5 µg/ml	1 µg/ml	0.5 µg/ml
	89.6 **41.9**	55.1 **33.6**	9.4 **11.7**	9.5 **3.6**
	40.6 **41.9**	18.5 **20**	9.4 **2**	0.6 **10**

Chalcones isolated from Licorice Roots and Derivatives thereof

Formula	10 µg/ml	5 µg/ml	1 µg/ml	0.5 µg/ml
	92 *98.0±0.0* 39.7±5.4	64 *98.0±0.0* 20.8±4.8	25 *69.0±4.0* 7.7±5.7	12 *39.0* 4.7±5.6
	18			
	91	28	12	

<u>Conclusion</u>

The data in Table 14.1 indicate the importance of oxygenation in the 2- or the 4-position or in the 2- and 4-positions for obtaining compounds which preferentially inhibit thymidine uptake into the parasites (with a single exception which is 2,5-dimethoxy-4′-allyloxychalcone; the high selectivity of 2,5-dimethoxy-4′-allyloxychalcone is interesting in view of the poor selectivity of 2,5-dimethoxy-4′-hydroxychalcone).

The *in vitro* results reported above confirm the hypothesis that the unsaturated α,β-position is of importance for the activity, c.f. the very low activity shown by 1-(4-hydroxyphenyl)-3-(2,4-dimethoxy)phenyl-2-propan-1-one. The pattern shown by the results indicates that one of the mechanisms of action might be an alkylation of the target biomolecule by the α,β-unsaturated ketones. This is substantiated by the reaction illustrated in Example 27, between licochalcone A and a thiol-containing peptide, where a nucleophilic thiol group is added to the α,β-double bond. The principle is well known from the anti-cancer activity of α-methylene sesquiterpene lactones. One such α-methylene sesquiterpene lactone has been tested in the *in vitro* model and has been found to be extremely active against *Leishmania* parasites *in vitro*, but at the same time also to show extreme toxicity on human lymphocytes. Thus, the substituents in the chalcone skeleton contribute to the selectivity of these compounds. The effect of the substituents can also be seen from the fact that chalcone in itself also has a very high activity against *Leishmania* parasites but is also extremely high toxicity against human lymphocytes, whereas, as is evident from the above data, e.g. 2,4- or 2- or 4-oxygen substituted chalcones show considerable selectivity for a number of chalcones. This selectivity seems to be hampered by the presence of a 5- or 3-substituent which, however, does not apply to the above-mentioned 2,5-dimethoxy-4′-allyloxychalcone.

EXAMPLE 15

Effect of Licorice extract and licochalcone A on *Plasmodium falciparum*

<u>Material and methods:</u>

Drugs. Licorice extract was obtained by extracting the comminuted licorice roots rich in licochalcone A with ethanol for 24 h, filtering the extract and concentrating the extract *in vacuo*, cf. Example 1.1.

***Plasmodium falciparum* continuous cultures.** *Plasmodium falciparum* was kept in continuous culture by a modification of the method originally described by Trager and Jensen (1976). Peripheral blood was drawn into 10 ml vaccutainers containing citra-

te-phosphate-dextrose, and stored at 4°C for 2-4 weeks before use. At the day of use, the cells were washed twice in RPMI medium (RPMI 1640 supplemented with 5% A Rh pos serum, Hepes 5.94 g/1000 ml medium, and sodium bicarbonate 7.5% (31 ml/1000 ml medium)), and after each wash the supernatant and the buffy coat containing leukocytes were removed. The parasites were cultured in Nunc culture flasks (Nunc, Roskilde, Denmark) containing 200 µl of packed erythrocytes in 5 ml of RPMI medium. The supernatants of the cultures were changed every 24 hours and the pack erythrocytes were supplied twice weekly. The parasitemia in the cultures was kept below 2%. The parasite cultures were grown at 37°C in an atmosphere of 2% oxygen, 5% carbon dioxide and 93% nitrogen. Two different parasite strains were kept in continuous culture: 1) The 3D7A chloroquine sensitive strain and 2) the chloroquine resistant DD2 strain. Both strains were kindly provided by Professor D. Walliker (Edinburgh, Scotland).

Testing of the effect of drugs and serum on the in vitro growth of the parasites. The experiments in which compounds were tested for their ability to inhibit parasite growth were performed by a modification of the method originally described by Jensen et al. (1982). Fifty µl of parasitized erythrocytes (parasitemia approximately 1%) in a concentration of $5x10^8$/ml and 50 µl of RPMI medium containing different concentrations of the test compound was added to each well of a 96 well flat-bottomed microtiter plate (Nunc). The cultures were then incubated for 48 hours, 24 hours before termination of the culture adding 20 µl of 3-H-hypoxanthine (40 uCi/ml) (New England Nuclear, Boston, MA, USA) was added to each well. The cultures were then harvested onto glass fiber filters using a Skatron cell harvester (Skatron, Lierbyen, Norway), and the incorporation of 3-H-hypoxanthine into the DNA of dividing parasites was determined by liquid scintillation spectrometry.

Control cultures with uninfected erythrocytes and infected erythrocytes in RPMI medium without test compounds were always performed in parallel to the test cultures.

In some experiments thin smears of parasite cultures were stained by Giemsa and examined under microscope (x 1000).

The test compounds described above, were diluted in RPMI medium immediately before use. In the experiments, chloroquine phosphate (Rigshospitalets Apotek, Copenhagen, Denmark) was used as a positive control as a drug known to inhibit parasite growth.

<u>Results</u>

The effect of licorice extract and licochalcone A on the uptake of 3-H-hypoxantine by dividing malaria parasites was tested by the addition of these compounds to *P. falciparum* cultures. Chloroquine was tested as a positive control.

Table 15.1 shows the effect of the compounds on a chloroquine sensitive parasite strain. It is apparent that both the extract and licochalcone A were able to inhibit the parasite growth. The concentration in which growth was retarded by 50% was between 0.5-1 µg/ml and 38-75 ng/ml for licochalcone A and chloroquine, respectively.

Table 15.2 shows the effect of the same compounds on a chloroquine resistent parasite strain. The compounds also inhibited the growth of this parasite strain. The concentration in which parasite growth was retarded by 50% was between 0.5-1 µg/ml and 75-150 ng/ml for licochalcone A and chloroquine, respectively. When comparing the effects of the compounds on the two parasite strains, it was clear that the effect of licochalcone A on the strains was comparable, whereas higher dosages of chloroquine were needed to inhibit the DD2 strain than the dosage needed to inhibit the 3D7A strain of the parasite.

These data are also shown in Fig. 4A and Fig. 4B.

Table 15.3 shows the effect of 4'-hydroxychalcone and 4-hydroxychalcone on the growth of the 3D7A strain of the parasite. The results indicate that these compounds were also able to inhibit parasite multiplication *in vitro*. The concentration in which growth was retarded by 50% was between 1-5 µg/ml for both compounds.

Table 15.4, 15.5, and 15.6 show the effect of 14 different licochalcone A analogues on the *in vitro* growth of *P. falciparum* (DD2 stain, chloroquine resistant strain). The analogues all inhibited the cultures in a dose dependant manner. The concentration in which the growth was inhibited by 50% was approximately between 1-10 µg/ml.

In some experiments the morphology of parasites cultured in the presence of licochalcone A was examined. In the cultures that had been incubated with licochalcone A in a concentration of 5 µg/ml, very few parasites could be detected inside the erythrocytes. The parasites that were found were pyknotic and without structure. In cultures incubated with licochalcone A at a concentration of 10 µg/ml, no parasites could be detected (data not shown).

Table 15.1. Effect of Licorice extract, licochalcone A and chloroquine on the *in vitro* growth of chloroquine-sensitive *P. falciparum* 3D7A strain. Data are given as percentage inhibition of the uptake of ^{3}H-hypoxanthine in cultures incubated with the test compound compared to the uptake in control cultures incubated with medium (mean±SEM).

Drug	Number of experiments	Growth in % of control (mean±SEM)
Licorice extract		
1:100	4	99.0±0.0001
1:200	4	96.0±2.0
1:400	4	63.0±6.0
1:800	4	32.0±5.0
Licochalcone A		
10 µg/ml	6	98.0±0.0001
5 µg/ml	6	98.0±0.001
1 µg/ml	6	69.0±4.0
0.5 µg/ml	6	39.0±6.0
0.1 µg/ml	6	15.0±3.0
Chloroquine		
300 ng/ml	6	98.8±0.3
150 ng/ml	6	97.1±1.0
75 ng/ml	6	80.2±3.7
38 ng/ml	6	34.0±6.1

Table 15.2. Effect of Licorice extract, licochalcone A and chloroquine on the *in vitro* growth of chloroquine-resistant *P. falciparum* DD2 strain. Data are given as percentage inhibition of the uptake of ^{3}H-hypoxanthine in cultures incubated with the test compound compared to the uptake in control cultures incubated with medium (mean ± SEM).

Drug	Number of experiments	Growth in % of control (mean±SEM)
Licorice extract		
1:100	4	98.0±2.0
1:200	4	87.0±9.0
1:400	4	65.0±14.0
1:800	6	26.0±4.0
Licochalcone A		
10 μg/ml	6	99.0±0.0001
5 μg/ml	6	98.0±1.0
1 μg/ml	6	66.0±3.0
0.5 μg/ml	6	41.0±4.0
0.1 μg/ml	6	5.0±2.0
Chloroquine		
300 ng/ml	4	94.8±3.0
150 ng/ml	4	80.3±4.1
75 ng/ml	4	43.9±3.1
28 ng/ml	4	11.3±4.2

Table 15.3. Effect of 4'-hydroxychalcone and 4-hydroxychalcone on the *in vitro* growth of chloroquine-sensitive *P. falciparum* 3D7A strain. Data are given as percentage inhibition of the uptake of ^{3}H-hypoxanthine in cultures incubated with the test compound compared to the uptake in control cultures incubated with medium (mean±SEM).

Drug	Number of experiments	Growth in % of control (mean±SEM)
4'-Hydroxychalcone		
10 µg/ml	3	95.5±1.1
5 µg/ml	3	64.2±8.5
1 µg/ml	3	40.5±11.1
0.5 µg/ml	3	29.9±10.7
4-Hydroxychalcone		
10 µg/ml	3	88.2±5.3
5 µg/ml	3	61.3±10.6
1 µg/ml	3	29.2±7.7
0.5 µg/ml	3	20.5±10.9

Table 15.4. Effects of analogues of licochalcone A on the *in vitro* growth of *P. falciparum* (DD2, chloroquine resistant strain). Data are given as percentage inhibition of the uptake of ^{3}H-hypoxanthine in cultures incubated with the test compound compared to the uptake in control cultures incubated with medium (mean±SEM).

Drug	Number of experiments	Growth in % of control (mean±SEM)
2,4-Dimethoxy-4'-allyloxychalcone		
10 µg/ml	9	75.2±7.8
5 µg/ml	9	44.3±10.5
1 µg/ml	9	23.6±11.2
0.5 µg/ml	9	29.1±10.8
2,4-Dimethoxy-4'-hydroxychalcone		
10 µg/ml	10	68.0±4.7
5 µg/ml	10	46.8±6.6
1 µg/ml	10	21.8±7.3
0.5 µg/ml	10	18.6±7.2
2,4-Dimethoxy-4'-methoxymethoxychalcone		
10 µg/ml	4	40.8±9.3
5 µg/ml	4	0±10.2
1 µg/ml	4	0±10.9
0.5 µg/ml	4	14.0±6.8
3,4-Dimethoxy-4'-hydroxychalcone		
10 µg/ml	7	82.6±4.7
5 µg/ml	7	48.5±8.4
1 µg/ml	7	7.1±8.1
0.5 µg/ml	7	9.3±9.2

Table 15.5. Effects of analogues of licochalcone A on the *in vitro* growth of *P. falciparum* (DD2, chloroquine resistant strain). Data are given as percentage inhibition of the uptake of ^{3}H-hypoxanthine in cultures incubated with the test compound compared to the uptake in control cultures incubated with medium (mean±SEM).

Drug	Number of experiments	Growth in % of control (mean±SEM)
3,4-Dimethoxy-4'-allyloxychalcone		
10 µg/ml	5	52.8±4.7
5 µg/ml	5	16.2±5.5
1 µg/ml	5	9.4±15.8
0.5 µg/ml	5	0±11.3
3,5-Dimethoxy-4'-allylchalcone		
10 µg/ml	5	79.1±10.5
5 µg/ml	5	44±8.1
1 µg/ml	5	3.4±4.7
0.5 µg/ml	5	9.1±5.2
2,5-Dimethoxy-4'-allyloxychalcone		
10 µg/ml	8	74.4±4.4
5 µg/ml	8	45.3±7.2
1 µg/ml	8	12.1±13.7
0.5 µg/ml	8	15.8±13.2
2,4-Dimethoxychalcone		
10 µg/ml	9	58.8±6.4
5 µg/ml	9	27.1±5.3
1 µg/ml	9	5.5±5.1
0.5 µg/ml	9	11±7.7

Table 15.6. Effects of analogues of licochalcone A on the *in vitro* growth of *P. falciparum* (DD2, chloroquine resistant strain). Data are given as percentage inhibition of the uptake of ^{3}H-hypoxanthine in cultures incubated with the test compound compared to the uptake in control cultures incubated with medium (mean±SEM).

Drug	Number of experiments	Growth in % of control (mean±SEM)
2,3-Dimethoxy-4'-allyloxychalcone		
10 µg/ml	9	49.9±9.1
5 µg/ml	9	22.2±10.1
1 µg/ml	9	29.6±10.9
0.5 µg/ml	9	28.5±9.6
2,3-Dimethoxy-4'-hydroxychalcone		
10 µg/ml	3	67.6±12.4
5 µg/ml	3	53.5±20.1
1 µg/ml	3	54.4±22.2
0.5 µg/ml	3	46±22
2,5-Dimethoxy-4'-hydroxychalcone		
10 µg/ml	8	92.5±2.4
5 µg/ml	8	68.4±6.8
1 µg/ml	8	21.2±11.1
0.5 µg/ml	8	14±7.5
2,6-Dimethoxy-4'-allyloxychalcone		
10 µg/ml	9	56.7±8.9
5 µg/ml	9	40.2±8.7
1 µg/ml	9	24.3±9.5
0.5 µg/ml	9	23.3±10.9

Table 15.7. Effects of analogues of licochalcone A on the *in vitro* growth of *P. falciparum* (DD2, chloroquine resistant strain). Data are given as percentage inhibition of the uptake of ^{3}H-hypoxanthine in cultures incubated with the test compound compared to the uptake in control cultures incubated with medium (mean±SEM).

Drug	Number of experiments	Growth in % of control (mean±SEM)
2,4-Dimethoxy-2'-hydroxychalcone		
10 µg/ml	10	55.3±7.4
5 µg/ml	10	33.7±8.2
1 µg/ml	10	20.8±8.8
0.5 µg/ml	10	23.1±8.3
2,6-Dimethoxy-4'-hydroxychalcone		
10 µg/ml	10	86.8±7.3
5 µg/ml	10	54.2±10.2
1 µg/ml	10	9±7
0.5 µg/ml	10	14.5±7.2

Conclusion

The results show that a certain licorice extract, licochalcone A and some other bis-aromatic α,β-unsaturated ketones are powerful inhibitors of nucleic acid biosynthesis in *Plasmodium falciparum* and are able to kill the parasite. There was no difference in the effect of licochalcone A on a chloroquine resistant and a chloroquine sensitive parasite strain and it must therefore be concluded that the compounds are as effective on chloroquine sensitive strains as they are on chloroquine resistant strains.

EXAMPLE 16

Effects of licochalcone A and some oxygenated chalcones on the *in vivo* growth of malaria parasites

Materials and Methods

Mice. BALB/c female mice aged eight weeks were used throughout.

Parasites. The Plasmodium sp. causing malaria in humans can only infect certain primates. Therefore it has not been possible to determine whether licochalcone A inhibits parasite multiplication of human malaria parasites *in vivo*. However, there are several Plasmodium sp. that infect rodents. These systems have earlier been used to test the ability of drugs to inhibit malaria infections *in vivo*. In the experiments described below mice were infected with either *P. yoelii* (strain not characterized, Table 15.1) or *P. yoelii* YM strain (Table 16.2 - 16.12) and were compared to the outcome of infection in untreated control animals and in animals treated with licochalcone A. The parasites were maintained by passage through BALB/c mice, and the animals were infected by injection of infected erythrocytes obtained from mice with a parasitemia of approximately 40%. The animals were injected intraperitoneally with either 2×10^5 (Table 16.1) or 1×10^6 (Tables 16.2 - 16.12) parasitized erythrocytes diluted in 0.9% NaCl and in a final volume of 0.2 ml. The day of infection was termed day 0.

Assessment of effect. The outcome of infection was assessed microscopically by examination of Giemsa stained blood films. The load of infection (the parasitemia) was calculated as the percentage of infected erythrocytes of the total number of erythrocytes.

Drug. Licochalcone A was prepared and stored as described in Example 8. Licochalcone A was administered intraperitoneally in a total volume of 0.2 ml at the indicated dosages. In parallel to the licochalcone A injections in the treated animals, control mice received injections of 0.2 ml 0.9% NaCl.

Table 16.1. Effect of licochalcone A in BALB/c mice infected with *P. yoelii* (strain not characterized). Parasitemia in 3 control mice (animal no. 1, 2, and 3 respectively, and mean) and in 3 mice treated with licochalcone A. The licochalcone A treatment was initiated 24 hours after infection with 2×10^5 parasites/mouse, and the mice treated with licochalcone A received 5 mg per kg body weight twice daily for 8 days.

Day	Control				Licochalcone A			
	No. 1	No. 2	No. 3	mean	No. 1	No. 2	No. 3	mean
D+1	0.01	0.02	0.02	0.02	0.05	0.03	0.02	0.03
D+2	1.0	2.0	1.6	1.5	0.5	0.05	0.01	0.2
D+3	6.0	3.0	2.5	3.8	2.0	0.5	0.5	1.0
D+4	11.0	8.0	6.0	8.3	4.0	1.5	2.0	2.5
D+5	15.0	12.0	13.0	13.3	4.0	1.5	2.0	2.5
D+6	22.0	18.0	17.0	19.0	4.5	2.0	1.0	2.3
D+7	25.0	27.0	22.0	25.0	4.0	1.5	1.0	2.2
D+8	37.0	33.0	30.0	33.3	4.0	1.5	1.5	2.3
D+9	45.0	40.0	44.0	43.0	4.0	1.0	1.5	2.2
D+11	59.0	55.0	52.0	55.3	2.0	1.0	2.0	1.7
D+12	died	59.0	64.0	61.5	2.0	1.0	2.0	1.7
D+14		61.0	61.0	61.0	1.0	0.0	1.0	0.7
D+16		died	61.0		0.0	0.0	0.1	0.03
D+18			40.0		0.0	0.0	0.0	0.0
D+20			45.0		0.0	0.0	0.0	0.0
D+21			died		0.0	0.0	0.0	0.0

Table 16.2. Effect of licochalcone A in BALB/c mice infected with *P. yoelii* strain YM. Parasitemia in 3 control mice (animal no 1, 2 and 3 respectively, and mean) and in mice treated with licochalcone A. The mice treated with licochalcone A received 10 mg per kg body weight twice daily from days +1 to +4.

Day	Control				Licochalcone A			
	No. 1	No. 2	No. 3	mean	No. 1	No. 2	No. 3	mean
D+2	0.1	0.1	2.0	0.73	0.1	0.1	0.01	0.1
D+3	1.0	1.5	2.0	1.5	0.00	0.5	0.01	0.2
D+4	9.0	14.5	18.0	13.8	0.00	1.2	0.00	0.4
D+5	45.0	61.0	62.0	56.0	0.1	1.5	0.00	0.7
D+6	90.0	91.0	81.0	87.3	1.0	4.0	1.5	2.2
D+7	91.0	died	died		2.0	4.0	3.0	3.0
D+8	died				4.0	8.0	8.0	6.7
D+9					20.0	18.0	31.0	23.0
D+10					27.0	24.0	died	25.5
D+11					33.0	41.0		37.0
D+12					80.0	68.0		74.0
D+13					80.0	20.0		50.0
D+14					65.0	5.0		35.0
D+15					2.0	2.0		2.0
D+16					0.5	0.1		0.3
D+17					0.0	0.0		0.0

Table 16.3. Effect of chloroquine on the parasitemia of mice infected with *P. yoelii* strain YM (10^6 parasites/mouse). Mice were 8 weeks old , female, BALB/c mice. Each group consisted of 5 mice. Chloroquine were given i.p.. The dosage, dosage interval and the days the animals were treated are indicated. Data are given as % parasitemia (mean±SEM) and were measured by microscopic counting of Giemsa stained blood smears, and mortality (no. of dead mice/total no. of tested mice). The treatment was initiated 3 h after infection.

Day	Control	Chloroquine 5 mg/kg/day once daily D0 - D+3	Chloroquine 2.5 mg/kg/day once daily D0 - D+3	Chloroquine 0.5 mg/kg/day once daily D0 - D+3
D+4	15.8±2.8	0.02±0.04	0.2±0.2	0.9±0.5
D+6	55.2±7.2	0	2.4±2.2	24±8.6
D+8	85.6±3.5 (5/5)	0.1±0.17	28.3±16.2	57.4±4.2
D+10		2.8±2.7	33.7±19.8	66.3±6.6 (2/5)
D+12		13±4.4	40.2±21	49.7±17.6 (2/5)
D+14		22.4±6.1	37.4±19.8	35.3±4.7 (2/5)
D+16		62.2±24.5	4.7±4.6 (2/5)	18.5±19.1 (3/5)
D+18		75.2±3.3	0 (2/5)	9 (4/5)
D+20		50±16.4 (1/5)	0 (2/5)	0 (4/5)

Table 16.4. Effect of licochalcone A in BALB/c mice infected with *P. yoelii* strain YM. Parasitemia in 2 control mice (animal no. 1 and 2) and in groups of 2 mice treated with licochalcone A at a dosage of 40 mg per kg body weight, 20 mg per kg body weight or 10 mg per kg body weight per day, respectively. The licochalcone A treatment was initiated 24 hours after infection, and the daily dosage was divided into 2 injections given of 12 hours interval. The drug was administered from days +1 to +6.

Day	Control		40 mg/kg/day		20 mg/kg/day		10 mg/kg/day	
	No. 1	No. 2	No. 1	No. 2	No.1	No. 2	No. 1	No. 2
D+4	45.0	26.0	0.1	0.5	0.01	0.0	1.5	4.0
D+5	89.0	68.0	0.5	1.0	0.0	0.0	1.5	3.0
D+6	died	90.0 died	1.0	1.5	0.0	0.0	2.0	2.5
D+7			4.5	5.8	0.01	0.05	8.0	10.0
D+8			12.0	15.0	1.0	1.5	18.0	20.0
D+10			32.0	38.0	5.0	8.0	39.0	42.0
D+11			51.0	50.0	15.0	19.2	67.0	71.0
D+12			76.0	81.0	23.0	26.0	85.0	89.0
D+13			89.0	88.0	12.0	15.0	90.0	90.0
D+14			died	died	5.0	8.0	died	died
D+16					0.1	0.5		
D+18					0.0	0.0		

Table 16.5. Effect of licochalcone A on the parasitemia of mice infected with *P. yoelii* strain YM (10^6 parasites/mouse). Mice were 8 weeks old female BALB/c mice. Each group consisted of 5 mice. Licochalcone A was given i.p. 3 h after infection and from D+1 to D+3. Data are given as % parasitemia (mean±SEM) and were measured by microscopic counting of Giemsa stained blood smears, and as mortality (no. of dead mice/total no. of tested mice).

Day	Control	15 mg/kg/day twice daily	10 mg/kg/day twice daily	5 mg/kg/day twice daily
D+4	32.2±2.2	2.0±2.0	1.1±1.0	1.6±0.6
D+6	86.4±2.8 (1/5)	10.4±7.4	14.2±4.6	13.8±4.4
D+8	(5/5)	22±11.6	30.2±6.4	36.6±5.7
D+10		22±11.6 (1/5)	48±5.4 (1/5)	64±5.0 (2/5)
D+12		4.8±3.2 (1/5)	82 (4/5)	(5/5)
D+14		0 (1/5)	(5/5)	

Table 16.6. Effect of licochalcone A on the parasitemia of mice infected with *P. yoelii* strain YM (10^6 parasites/mouse). Mice were 8 weeks old female BALB/c mice. Each group consisted of 5 mice. The dosage, dosage interval and the number of days the animals were treated are indicated. Data are given as % parasitemia (mean±SEM) and were measured by microscopic counting of Giemsa stained blood smears, and as mortality (no. of dead mice/total no. of tested mice).

Day	Control	Licochalcone A 3 h after infection From D+1 to D+5	
		15 mg/kg/day twice daily	10 mg/kg/day twice daily
D+6	70±4.5	0	0.1±0.1
D+8	80 (4/5)	0	0.7±0.4
D+10	(5/5)	0	6.0±3.8
D+12		0	14.4±8.9
D+14		0	17±17 (1/5)
D+16		0	0 (2/5)
D+18		0	0 (2/5)

Table 16.7. Effect of licochalcone A in BALB/c mice infected with *P. yoelii* strain YM. Parasitemia in 2 control mice (animal no. 1 and 2) and in 2 groups of 2 mice treated with licochalcone A. Group 1 received one injection of licochalcone A (10 mg per kg body weight) 3 hours before infection. Group 2 received one injection of 10 mg per kg body weight 1 hour after infection, and 2 injections of 10 mg per kg body weight on days +1 and +2, respectively.

Day	Control		10 mg/kg[a]		10 mg/kg[b]	
	No. 1	No. 2	No. 1	No. 2	No.1	No. 2
D+4	25.0	20.0	1.0	2.0	0.1	0.1
D+5	73.0	61.0	5.0	6.0	0.1	0.5
D+6	died	88.0 died	22.0	21.0	0.5	0.5
D+7			55.0	45.0	1.5	0.8
D+8			65.0	15.0	0.1	0.1
D+9			85.0	82.0	0.1	0.1
D+10			died	90.0	0.00	0.00

[a] Intraperipetoneally 3 hours before infection
[b] Intraperipetoneally 1 hour after infection; two times per day on D+1 and D+2.

Table 16.8. Effect of licochalcone A in mice infected with *P. yoelii* strain YM. Mice were 8 weeks old female BALB/c mice. Each group consisted of 5 mice. Licochalcone A was given i.p.. The dosage interval and the number of days the animals were treated are indicated. Data are given as % parasitemia (mean±SEM) and were measured by microscopic counting of Giemsa stained blood smears, and as mortality (no. of dead mice/total no. of tested mice).

Day	Control	Licochalcone A 10 mg/kg twice daily D-1, D0	Licochalcone A 10 mg/kg four times daily D+4 to D+7
D+4	15.8±2.8	1.2±1.1	14.2±3.8
D+6	55.2±7.2	9.7±0.7	6.6±1.5
D+8	85.6±3.5 (5/5)	27.8±16.1	17±6.2
D+10		47.4±27.7	27.6±12.1
D+12		55.7±48.4 (2/5)	50.4±15.6
D+14		0 (4/5)	58.6±9.0
D+16		0 (4/5)	75.8±6.6 (1/5)
D+18		0 (4/5)	53±25 (2/5)
D+20		0 (4/5)	19±9.7 (3/5)

Table 16.9. Effect of licochalcone A on the parasitemia of mice infected with *P. yoelii* strain YM. Mice were 8 weeks old, 20 g body weight, female BALB/c mice. Licochalcone A was given by intraperitoneal implantation of 1007D Micro-Osmotic Pump (6 mg/100 µl/mouse) 3 h after infection. The pump releases 0.5 µl of drug solution per hour. The drug was released during a period of 7 days. Data are given as % parasitemia and were measured by microscopic counting of Giemsa stained blood smears.

Day	unoperated	DMSO		Licochalcone A	
		mouse 1	mouse 2	mouse 1	mouse 2
D+7	30	26	15	0.5	0
D+9	78 (died D+10)	72	59	6	0

Table 16.10. Effect of licochalcone A on the parasitemia of mice infected with *P. yoelii* strain YM (10^6 parasites/mouse). Mice were 8 weeks old, 20 g body weight, female BALB/c mice. Licochalcone A was given orally (100 mg/kg/dose) 3 and 24 h after infection. Data are given as % parasitemia (mean±SEM) and were measured by microscopic counting of Giemsa stained blood smears.

Day	Control				Licochalcone A			
	No.1	No. 2	No. 3	Mean	No. 1	No. 2	No. 3	Mean
D+4	17	20	22	19.7	0.5	1.0	1.5	1.0
D+7	died	died	died		4.0	10.0	15.0	9.7
D+10					31.0	55.0	died	43
D+13					5.0	8.0		6.5
D+16					0	0		0

Table 16.11. Effect of 2,4-dimethoxy-4'-hydroxychalcone (2,4m4'hc) and 2,4-dimethoxy-4'-allyloxychalcone (2,4m4'ac) on the parasitemia of mice infected with *P. yoelii* strain YM (10^6 parasites/mouse). Mice were 8 weeks old female BALB/c mice. The analogues were given i.p.. The dosage, dosage interval and the number of days the animals were treated are indicated. Data are given as % parasitemia (mean±SEM) and were measured by microscopic counting of Giemsa stained blood smears and as mortality (no. of dead mice/total no. of tested mice).

Day	Control	2,4m4'hc 3 h after infection twice daily From D+1 to D+5		
		40 mg/kg	30 mg/kg	20 mg/kg
D+6	70±4.5	(5/5)	46.4±2.1	4.8±1.5
D+8	(5/5)		70.8±3.5 (1/5)	12.4±2.4
D+10			(5/5)	58.6±14
D+12				25 (3/5)
D+14				6 (3/5)
D+16				0 (3/5)

Table 16.11 continued.

Day	Control	2,4m4'ac 3 h after infection twice daily From D+1 to D+5		
		40 mg/kg	30 mg/kg	20 mg/kg
D+6	70±4.5	55.8±1.5	37.4±0.9	0.4±0.2
D+8	(5/5)	83±2.1 (2/5)	67.2±0.9	0.8±0.2
D+10		(5/5)	(5/5)	14.3±4.0
D+12				32.4±4.0
D+14				7 (3/5)
D+16				0 (3/5)

Table 16.12. Effect of 2,4-dimethoxychalcone (2,4mc) and 2,5-dimethoxy-4'-allyloxychalcone (2,5m4'ac) on the parasitemia of mice infected with *P. yoelii* strain YM (10^6 parasites/mouse). Mice were 8 weeks old female BALB/c mice. The analogues were given i.p.. The dosage, dosage interval and the number of days the animals were treated are indicated. Data are given as % parasitemia (mean±SEM) and were measured by microscopic counting of Giemsa stained blood smears and as mortality (no. of dead mice/total no. of tested mice).

Days	Control	2,4mc twice daily			
		40 mg/kg	30 mg/kg	20 mg/kg	10 mg/kg
D+5	80±11	78 (4/5)			
D+6	89 (4/5)	(5/5)	29 (3/5)	85 (4/5)	(5/5)
D+8	(5/5)		15 (4/5)	(5/5)	
D+10			65 (4/5)		
D+12			(5/5)		

Table 16.12 continued.

Days	Control	2,5m4'ac twice daily			
		40 mg/kg	30 mg/kg	20 mg/kg	10 mg/kg
D+5	80±11				
D+6	89 (4/5)	(5/5)	62 (3/5)	79 (4/5)	(5/5)
D+8	(5/5)		78 (4/5)	(5/5)	
D+10			(5/5)		
D+12					

Results and discussion

P. yoelii is a very virulent parasite in mice. The parasitemia increased rapidly and the animals die if treatment is not initiated. The control animals of the experiment presented in Table 16.1 reached a parasitemia of approximately 50% at day 11, and the animals died between day 12 and 21. The control animals of the experiments presented in Table 16.2 - 16.12 had an even more rapid disease development, these animals reached a parasitemia of 60 - 70% on day 5, and the animals died on day 6, 7 or 8. The experiment reported in Table 16.1 was performed with another strain of parasites than the following experiments, this is probably the reason for the slight difference in the disease development between this experiment and the rest of the experiments.

Table 16.1 and 16.2 show that licochalcone A given two times daily in a dosage of 5 - 10 mg per kg body weight maintained the parasitemia at a low level for as long as the treatment was given. After the treatment was withdrawn, the parasitemia increased, but the delay of parasitemia caused by the licochalcone treatment enabled most animals to control the infection and clear the parasites.

The results presented in Table 16.3 show the effect of chloroquine treatment in mice infected with the virulent *P. yoelii* YM infection.The data show that treatment with chloroquine in this model is unable to protect the animals from parasitemia, and underlines the difficulty of drug treatment in this model. When interpreting the efficiency of the test compounds this fact should be taken into consideration.

The results presented in Table 16.4 confirm the results presented in Table 16.1 and 16.2. Furthermore they show that a dosage of 20 mg per kg body weight per day was more effective than a dosages of 10 mg per kg body weight per day or 40 mg per kg body weight per day, when the drug was administered twice daily. This indicates that to increase the efficacy of the drug it should be attempted to decrease the intervals between the injection of the drug rather than increasing the amount of drug per injection.

Table 16.5 shows the results of an experiment in which licochalcone A treatment was maintained for the same period as chloroquine in the experiments reported in Table 16.3. The results indicate that the efficiency of licochalcone A was comparable to that of chloroquine. The experiments reported in Table 16.6 were essentially performed as the experiments reported in Table 16.5, the only difference was that the treatment was continued for five days instead of three days. The results (Table 16.6) indicate that the efficiency of licochalcone A was markedly enhanced by extending the treatment period.

The results presented in Table 16.7 show that one injection of 10 mg per kg body weight 3 hours before infection causes a considerable delay in the increase of parasitemia (group 1). This is interesting since the pharmaco-kinetic studies indicated that the half-life of the drug in the plasma was about 20 min. Hence, the results indicate that the drug was concentrated within the erythrocytes or that metabolites of the drug could affect the parasites. Table 16.7 also showed that the efficiency of the drug was higher if the drug was given shortly after infection, since mice in which licochalcone A treatment was initiated 1 hour after infection controlled the infection even when the treatment was given for 2 days only (group 2). When the drug was given the day before infection and the day of infection at a dosage of 10 mg two times daily (Table 16.8), the efficiency of the drug was comparable to that found in Table 16.7 (group 1). The experiment reported in Table 16.8 was performed to test the efficiency of the compound in animals in which the infection was established and the parasitemia was high at the initiation of treatment. The results show that licochalcone A was able to reduce the parasitemia as long as the treatment was maintained. However, when the treatment was stopped, the parasitemia increased.

Table 16.9 shows the results of an experiment in which licochalcone A was injected into a pump from which the drug was slowly released over a period of 7 days. Administered this way 6 mg of licochalcone A seems to have considerable efficiency.

Table 16.10 shows the results of experiments where licochalcone A was given orally. Due to difficulties in administering the drug via a mouth catheter, the drug was only administered twice. The results show that licochalcone A administered orally has the same efficiency as when administered by i.p. injections.

Tables 16.11 and 16.12 show the results of studies in which 4 licochalcone A analogues were tested in the *in vivo* mouse model. The analogues presented in Table 16.12 did not have any effect. The analogues presented in Table 16.11 inhibited the parasitemia when given at a dosage of 60 mg/day or 40 mg/day, respectively. When administered at a dosage of 80 mg/day, no effect was seen. At a dosage of 40 mg/day, the analogues were able to prevent the death of 2 out of 5 animals.

Conclusion

One strain of *P. yoelii* with a parasitemia up to 61% are able to kill the control mice within 12-20 days following infection. Using a more virulent strain of *P. yoelii* malaria called YM, the control mice died within 6-7 days following infection with a parasitemia of up to 90%.

The *in vivo* experiments using *P. yoelii* infection in mice indicate that administration of licochalcone A at concentrations of 15 mg per kg body weight twice a day (30 mg per kg body weight per day) over a period of 5 days was able to completely clear the parasite from the infected mice and completely protect the mice from death induced by the parasite as compared to the control mice.

If the treatment with licochalcone A was initiated one hour before the animals were infected two days of treatment with 20 mg licochalcone A per day were sufficient to control the parasitemia and prevent death of the animals (Table 16.7, group 2).

The data shown in Table 16.4 using the same strain of *P. yoelii* YM show that the ranges of concentrations of licochalcone A used for the treatment of these animals were fairly narrow, indicating that concentration of 10 mg per kg body weight (20 mg per kg body weight per day) given twice a day was able to completely protect the mice as shown in the previous experiment. On the other hand, the third group of animals receiving a concentration of 5 mg per kg body weight (10 mg per kg body weight per day) given twice a day over a period of 6 days were not able to survive the infection.

In the experiments reported in Tables 16.3 and 16.5, chloroquine (a widely used anti-malarial drug) and licochalcone A were administered at different dosages but with the same regimen with regard to administration route, dosage intervals and length of the treatment. The results indicate that the efficiency of the two compounds was comparable.

The experiment shown in Table 16.7 (group 1) using the same malaria parasite showed that when licochalcone A was given three hours before the infection and only once in a dose of 10 mg per kg body weight, the parasitemia was lower in the treated group in the first week of infection than in the controls. However, the treatment did not prevent the death of the animals.

The experiment reported in Table 16.8 was performed to test the efficiency of the compound in animals in which infection was established and in which the parasitemia was high when the treatment was initiated. The results show that licochalcone A was able to reduce the parasitemia as long as the treatment was maintained. However, when the treatment was stopped, the parasitemia increased.

Table 16.9 shows the results of an experiment in which licochalcone A was injected into a pump from which the drug was slowly released over a period of 7 days. Administered this way 6 mg of licochalcone A seems to have considerable efficiency. This is an interesting observation since it indicates that the efficiency of licochalcone A is not dependent on the high concentration of the compound which may be as-

sumed to follow i.p. injections.

Table 16.10 shows the effect of licochalcone A when administered orally. Although the results are preliminary, they indicate that the efficiency of licochalcone A administered orally is at the level of the efficiency when administered by i.p. injections.

Four licochalcone A analogues were tested in the *in vivo* mouse model. The analogues presented in Table 16.11 inhibited the parasitemia when given at a dosage of 60 mg/day or 40 mg/day, respectively. When administered at a dosage of 80 mg/day, no effect was observed. At a dosage of 40 mg/day, the analogues were able to prevent the death of 2 out of 5 animals.

Conclusion

Thus, the data presented in the 12 tables indicate that:

1. Licochalcone A is able to completely clear the infection of *P. yoelii* in mice.

2. The dose range protective for the mice is fairly narrow when the drug is administered i.p..

3. When licochalcone A is given one hour after infection of the mice, the compound is able to completely stop or inhibit the multiplication of the parasite and establishment of the infection in these mice, indicating that the compound can be used for prophylactic measures. When the compound is used for the treatment of an established infection, it is able to decrease the parasitemia. This is important because it allows the mice to establish an immune response which then may be able to eventually eliminate the parasites. Because of the short half-life of licochalcone A, it appears advantageous either to administer the compound frequently or to administer the compound in a slow release composition or as a prodrug from which the drug is slowly released.

4. Licochalcone A is effective when administered orally.

5. Analogues of licochalcone A has some effect in the *in vivo* mouse model, although the efficiency of the tested analogues appears to be less than the efficiency of licochalcone A.

EXAMPLE 17

Effect of licochalcone A on *Legionella* and some other bacterial species

Materials and methods:

Drug. Licochalcone A was isolated as described in Example 1.

Bacteria. 20 *Legionella* strains: Five clinical isolates from bronchial secretions and a lung abscess: 2 *Legionella pneumophila* serogroup 1 and 3 *Legionella micdadei* (*L. detroit*, *L. bari*, *L. F 1433*). Eight *Legionella pneumophila* serogroups 1-7 and one strain of each of *L. bozemanii*, *L. dumoffii*, *L. gormanii*, *L. micdadei*, *L. feelei*, *L. wadsworthii*, *L. longbeacheae*. *Staphylococcus aureus* ATCC 25923 was the control strain.

The following respiratory commensals were tested: three *Corynebacterium* species, two *Branhamella catarrhalis*, one *Streptococcus pneumonia*, one *Non-haemolytic streptococci*, one *Bacillus subtilis*, one *Sarcina lutea*. All strains were kept frozen at -80°C until assayed.

The *Legionella* strains were subcultured on buffered charcoal yeast extract with alfa-ketoglutarate (BCYE-α), and the rest of the strains were subcultured on 10% horse blood agar for 48 hours and 24 hours, respectively.

Minimal inhibitory concentrations. Macrodilution rows were made with buffered yeast extract with alfa-ketoglutarate (BYE-α) with 2 ml aliquots in vials, the dilution of G.radix extract from 1000 µg/ml to 0.04 µg/ml. Suspensions of Legionella species and the other pathogens and commensals were made in BYE-α. All the dilution rows were inoculated to give a final concentration of 10^5 CFU/ml. After incubation at 37°C for 2 and 24 hours, respectively, aliquots of 10 µl were taken from all dilution steps and plated onto BCYE-x agar plates (all *Legionella* species) and to 10% horse blood agar (all *non-Legionella* strains). All the BCYE-x plates were incubated for 48 hours in a humid atmosphere at 37°C and read. The inoculated 10% horse blood agar plates were incubated in a normal atmosphere at 37°C for 24 hours and read.

Results

All the clinical *Legionella pneumophilia* isolates were sensitive to licochalcone A, their MIC-values ranging from 1 to 4 µg/ml, whereas *Legionella gormanii* and the 4 *L. micdadei* isolates had MIC-values from 15 to 500 µg/ml.

The Gram positive cocci all had MIC's from 4 to 8 mg/ml. One of the corynebacterium

species was very sensitive, having MIC of 0.3 μg/ml.

Table 17.1. *Legionella* species susceptibility to licochalcone A in μg/ml.

	No. of strains	MIC
L. pneumophilia serogr. 1	4	1-4
L. pneumophilia serogr. 2-7	6	2-4
L. bozemanii	1	2
L. domoffii	1	2
L. gormanii	1	500
L. micdadei	1	500
L. micdadei (*Detroit*)	1	15
L. micdadei (*Bari*)	1	60
L. micdadei (*F 1433*)	1	60
L. feelei	1	4
L. wadsworthii	1	2
L. longbeacheae	1	1

Table 17.2. Susceptibility of Gram positive pathogens and commensals to licochalcone A in μg/ml.

	No. of strains	MIC
Staphylococcus aureus ATCC 25923	18	
Sarcina lutea	1	4
Non-haemolytic streptococci	1	4
Streptococcus pneumonia	1	4
Corynebacterium species	3	0.3-4
Bacillus subtilis	1	4

Conclusion

Licochalcone A exhibited a clear anti-legionella activity at MIC values form 1 μg/ml, in most cases from 1 to 4 μg/ml.

The low MIC for *L. pneumophila*, the human pathogen, is promising and therefore licochalcone A can be considered as a potential drug against respiratory infections.

The reason that the MIC was very high for the inhibition of *Legionella micdadei* could be that the cell wall of *L. micdadei* is different from the cell wall of *L. pneumophila* (Hébert et al, 1984) and therefore, that the uptake of licochalcone A in *L. micdadei* is poorer than the uptake in *L. pneumophila* .

Although some of the Gram positive pathogens and commensals were sensitive to licochalcone A, it was surprisingly found that not all bacteria even within the same group of bacteria were sensitive to the licochalcone A in atoxic concentrations. However, the bacteria showing sensitivity towards licochalcone A were the pathogenic bacteria.

EXAMPLE 18

Effect of licochalcone A on *Helicobacter pylori*

<u>Material and methods</u>

Bacteria. 16 recent clinical isolates of *Helicobacter pylori* from patients with duodenal ulcer or chronic gastritis. The medium used both for transportation of the biopsies and the minimal inhibitory concentration (MIC) assays was: brain heart infusion broth with 0.0002% resazurine, 0.15% L-cystein and 5% horse serum (Helicomedia), pH 6,8.

Staphylococcus aureus ATCC 25923 was used as control strain. The *Helicobacter pylori* strains were subcultured on chocolate agar plates with cysteine in a microaerophilic atmosphere at 37°C for 72 hours and kept frozen at -80°C until assayed.

MIC determinations. Macrodilution rows were made with Helicomedia with 2 ml in all vials. The dilution of licochalcone A was from 500 µg/ml to 1 µg/ml.

All dilution rows were inoculated to give a final concentration of 10^5 CFU/ml.

The MIC rows were incubated under microaerophilic conditions for two hours. Hereafter aliquots of 10 µl were taken from all dilution steps and plated on chocolate agar with cysteine and incubated in a microaerophilic atmosphere for 72 hours at 37°C. The control strain *Staphylococcus aureus* was incubated both aerobically and microaerophilically at 37°C for 24 hours. All plates were read by the Karber method.

Results

Table 18. The minimal inhibitory concentration of licochalcone A on *Helicobacter pylori* in µg/ml.

MIC_{50}	MIC_{90}	Range
62	125	16-125

Only one strain had a low MIC: 16 µg/ml.

After 2 hours incubation with licochalcone A, the MIC of *Staphylococcus aureus* was, as expected, 4 µg/ml when incubated in a normal atmosphere, whereas under microaerophilic conditions it raised to more than 500 µg/ml.

Conclusion

From the results it is seen that very high concentration of licochalcone are necessary to inhibit bacteria under microaerophilic conditions. However, since the use of licochalcone A and other bis-aromatic α,β-unsaturated ketones against *Helicobacter pylori* would be in the treatment or prophylaxis of gastric ulcer which often is caused or aggravated by *Helicobacter*, it means that the treatment would be local treatment, such as any suitably targeted controlled release compositions and therefore it is possible to administer high doses.

EXAMPLE 19

Effect of licochalcone A on *Mycobacteria* species

Materials and methods

63 strains of mycobacteria were used in this study. The bacteria were grown in Dubos broth media before susceptibility testing. Licochalcone A was isolated as described in Example 1. Licochalcone A was dissolved in dimethyl sulfoxide (DMSO) and diluted in distilled water to the desired concentration.

Susceptibility testing was performed radiometrically by using a BECTEC 460-TB apparatus (Becton Dickinson) in a confined atmosphere (5% CO_2). Bacterial growth was measured as a function of the ability of the bacteria to catabolize ^{14}C-labelled palmitic acid in the BECTEC 7H12B TB medium (Becton Dickinson) during growth,

which resulted in the release of ^{14}C-labelled CO_2. The growth was expressed as a numerical value called the growth index (GI) which ranged from 1 to 999. The 7H12 vials were inoculated with 0.1 ml of an appropriately diluted Dubos broth culture to give a final inoculum of about $5x10^4$ colony-forming units (CFU) per ml together with 0.1 ml of different concentrations of licochalcone A. The final concentrations of licochalcone A tested ranged from 1.25 μg/ml to 80 μg/ml. A vial without licochalcone A, but with an inoculum diluted 1:100, was included as a control. The final inoculum was determined by culturing 0.1 ml from the control vial onto one Lowenstein-Jensen slant. The vials were incubated under stationary conditions at 35°C and growth was monitored by daily GI determination for 7 days. At day 7, 0.1 ml from each vial with a GI reading < 30 was cultured onto one Lowenstein-Jensen slant. Colony counts were enumerated after incubation at 35°C for 3 weeks.

Minimal inhibitory concentration (MIC) was defined as the lowest concentration of licochalcone A which could inhibit 99% or more of the mycobacteria population.

Minimal bactericidal concentration (MBC) was defined as the lowest concentration of licochalcone A which killed 99% or more of the mycobacteria population.

Results

Table 19.1. Eighteen different species of *Mycobacteria* were screened for susceptibility of 20 μg/ml of licochalcone A.

Species MIC≤20 μg/ml	 MIC>20 μg/ml
M. tuberculosis	*M. szulgai*
M. bovis	*M. avium/intracellular*
BCG	*M. scrofulaceum*
M. kansasii	*M. malmoense*
M. xenophii	*M. terrae/triviale*
M. marinum	*M. nonchromogenicum*
	M. smegmatis
	M. flavescens
	M. fortuitum
	M. chelonae

Determinations of MIC and MBC of licochalcone A against strains belonging to the *M. tuberculosis* complex:

M. tuberculosis	$mean_{MIC}$ = 7.1 μg/ml
	$range_{MIC}$ = 5-10 μg/ml (n=19)
	$mean_{MBC}$ = 40 μg/ml (n=2)
M. bovis	$mean_{MIC}$ = 15.7 μg/ml
	$range_{MIC}$ = 10-20 μg/ml (n=8)
BCG	$mean_{MIC}$ = 8.6 μg/ml
	$range_{MIC}$ = 5-10 μg/ml (n=3)
	$mean_{MBC}$ = 40 μg/ml (n=3)

Determinations of MIC of licochalcone A against strains of *M. avium/intracellular*:

M. avium (AIDS patients): $mean_{MIC}$>80 μg/ml (n=4)
M. avium (non AIDS patients): $mean_{MIC}$>80 μg/ml (n=7)
M. intracellular: $mean_{MIC}$ = 50.0 μg/ml
$range_{MIC}$ = 20-80 μg/ml (n=9)

Table 19.2. Influence of 10% serum on MIC determination of licochalcone A.

MIC (μg/ml) with and without 10% Human Serum: Strain=H37RV

	Ethambutol (40% protein binding)	Ofloxacin (5% protein binding)	Fusidic acid (90% protein binding)	Licochalcone A
MIC_{-serum}	1	0.5	8	5
MIC_{+serum}	2	0.5	32	40

Conclusion

With a proposed "cut off" concentration value of 20 μg/ml, most strains belonging to the *M. tuberculosis* complex were susceptible. The bactericidal concentration was 4 to 8 times the inhibitory concentration which in all strain tested was higher than the "cut off" concentration.

All *M. avium/intracellular* strains were on the other hand resistant with MIC ≥20 µg/ml and most with MIC >80 µg/ml.

From Table 19.2 it is seen that MIC of licochalcone A increases 8-fold when supplemented with 10% of serum which may indicate that licochalcone A is highly protein-bound.

EXAMPLE 20

Licochalcone A absorption studies

The absorption pattern and pharmaco-kinetics of licochalcone A in rats given by oral route were determined compared with pharmacokinetics of licochalcone A in rats given intravenously.

Number of rats: 4 for each group

Groups:	1. receiving licochalcone A
	2. receiving buffer

Dose :	1000 mg/kg body weight of licochalcone A.
Route:	Oral, administered once.
Samples:	2 ml of blood was drawn 4 times from each rat.
1.	4 hrs after licochalcone A administration
2.	24 hrs " " "
3.	48 hrs " " "
4.	one week " " "

After one week one rat from each group was sacrificed and the spleen and liver were examined for histology and licochalcone A measurements.

Number of rabbits: 1 rabbit for each group

Groups:	1. receiving licochalcone A
	2. receiving buffer

Dose:	20 mg per kg body weight
Route:	Intravenously, administered once
Samples:	Samples were drawn from each rabbit twice.
1.	1 hour after licochalcone A administration
2.	24 hours after licochalcone A administration

Results

In the serum samples of rats receiving 1000 mg per kg body weight taken 4 hours after licochalcone A administration, licochalcone A concentrations of 0.14 μg/ml and 0.16 μg/ml were detected. Licochalcone A concentrations in the serum after 24 hours were <0.05 μg/ml (detection limit of the assay). It should be mentioned that licochalcone A absorbs to glassware and perhaps other material during the assay, and therefore the actual serum concentrations may be higher.

In rabbits, concentrations of 0.35 μg/ml licochalcone A were detected in the serum one hour following intravenous administration of 20 mg per kg body weight of licochalcone A. 24 hours after administration the licochalcone A concentration in the serum was < 0.05 μg/ml (detection limit of the assay).

Conclusion

From the results it is seen that already after 4 hours, the concentration of licochalcone A in the blood was very small compared to the dose administered orally to the rats. This could be due to an extraordinarily large first pass elimination of licochalcone A in the liver since the concentration found in rabbits 1 hours after intravenous administration was higher even though the dose administered was lower than in the rat study.

EXAMPLE 21

Animal toxicity studies with licochalcone A

Study in mice

Drug. Licochalcone A solution was prepared as follows: 20 mg of licochalcone A was dissolved in 0.2 ml of ethanol. 60 mg of melted polyoxyethylene (23)laurylether (Brij 35) was added. 1.7 ml of phosphate buffer was then added while stirring on a warm plate. pH in the licochalcone A solution was adjusted to 7.3.

Mice. NMRI female mice (30-35 gram) in groups of 4 each were used for these studies. For the oral route experiment, the animals were fasted for 16 hrs with access to drinking water. Licochalcone A was suspended in 2% carboxymethylcellulose in water. This suspension was available for the mice in an amount of 0.1 ml per 10 gram body weight. The animals were observed every day for 7 days. For the intravenous injection experiment the animals were not fasted. Licochalcone A in solution (20

mg/ml) prepared as mentioned above was injected intravenously 0.1 ml per 10 gram body weight over a period of one hour and again 24 hrs later.

Results

Table 21.1. Licochalcone A toxicity studies in mice.

Licochalcone A dose (mg/kg)	Solution strength	No. dead (after 1 hr)
Control Brij	3	0
Licochalcone A:		
100	1	4
60	0.6	4
50	0.5	1
20	0.2	0
1000 mg/kg perorally	10	0 (after 7 days)

Approximate LD50 i.v. = 55 mg per kg body weight
LD50 peroral > 1000 mg per kg body weight

Study in rats. To 4 rats was administered one dose of 1000 mg per kg body weight perorally. The rats were observed for one week. None of the animals died.

Results

LD50 peroral in rats > 1000 mg per kg.

Conclusion

The results above shows that licochalcone A is atoxic even in high concentrations.

EXAMPLE 22

Animal toxicity studies of 4-hydroxychalcone in mice

Mice. BALB/c female mice, 8 weeks old, 20 g body weight.

Drug. 4-Hydroxychalcone prepared as in Example 2.

LD50. 80 mg of 4-hydroxychalcone was dissolved in 0.2 ml of 99% (v/v) ethanol, and then mixed with 240 mg of Brij 35 dissolved in 19.8 ml of buffer pH 7.3 ($NaH_2PO_4 \cdot H_2O$ 26 mg, $Na_2HPO_4 \cdot 2H_2O$ 520 mg and distilled water 100 ml), and then sterile filtered through a 0.22 µm Millipore filter. Drug was injected intraperitoneally into mice once.

Results

Table 22.1. 4-Hydroxychalcone toxicity study in mice.

Groups	n	No. died after 24 h
50 mg/kg i.p.	4	0
100 mg/kg i.p.	4	0
200 mg/kg i.p.	4	1
400 mg/kg i.p.	4	2

Conclusion

From Table 22.1 it is seen that when administering intraperitoneally, LD50 is 400 mg per kg. However, intraperitoneal administration is comparable to intravenous administration and then 400 mg per kg is a very high dose, which means that in therapeutical doses licochalcone A is an atoxic drug.

EXAMPLE 23

Quantification of licochalcone A in serum or plasma

Serum or plasma was diluted with one part of acetonitrile, left for 15 min at 4°C, and centrifugated for 3 min at 10,000 g. An aliquot of the supernatant was chromatographed at 37°C by HPLC over Spherisorb ODS-2 (5 µm, 120 X 4.5 mm, Phase Separation LTD, UK) using acetonitrile aqueous acetic acid, 2%, (1:1) as an eluent, flow rate 1.5 ml/min, and UV detection (370 nm and 254 nm).

Detection limit (25% acetonitrile in water, 370 nm): 0.05 µg/ml.
Detection limit (plasma, 370 nm): 0.1 µg/ml

t_R is approximately 3.23 min for licochalcone A (k' 3.51) and 3.93 min for 2,4-dimeth-

ylnitrobenzene (k' 8.32) used as an internal standard.

No interfering peaks were observed in serum or plasma from rodents not treated with licochalcone A.

Conclusion

This quantification test will be used in the pharmaco-kinetic studies of bis-aromatic α,β-unsaturated ketones and derivatives thereof (see Examples 24 and 25).

EXAMPLE 24

Quantification of intracellular concentration of licochalcone A

Material and Methods

UV-detector: Beckmann, model G, 2400 (single-ray apparatus)

Centrifuge: Heraeus, Christ, Labofuge GL.

Methods. A standard curve of licochalcone A solutions in concentrations between 0.5 µg/ml and 10 µg/ml was prepared. A basic solution of 10 mg licochalcone A in 5 ml DMSO was produced. The desired concentrations was obtained by dilution of the basic solution with Krebs Ringer solution. The solutions were measured with a UV-single-ray apparatus at 385 nm. The detection wavelength was determined by UV-scanning of licochalcone A dissolved in DMSO and Krebs Ringer solution.

The uptake of licochalcone A in human cells was determined by using granulocytes (PMN) and mononuclear cells (MNC) incubated with 4 different concentrations of licochalcone A at 37°C. Cells incubated without licochalcone A, as well as licochalcone A solutions without cells were used as control measurements.

Isolation of the different cell types from blood was done as described in Fig. 7.

The isolated cells were counted in microscope and diluted with Krebs Ringer solution to 2×10^4 cells/ml and 4×10^4 cells/ml.

To supernatants of 2×10^4 cells/ml and 4×10^4 cells/ml were added aliquots of the licochalcone A solution to give final concentrations of 20, 10, and 5 µg/ml. After incubation for 5 min at 37°C the cells were spun down at 2000 rpm for 10 min. The supernatant was collected and the licochalcone A concentration determined spectro-

photometrically.

The pellet was resuspended in distilled water and lysed by freezing and thawing several times. The suspension was centrifugated at 1500 rpm for 10 min and the licochalcone A concentration determined spectrophotometrically.

Results

The amount of licochalcone A taken up by the cells varied between 10% and 25%.

Conclusion

Licochalcone A is taken up by the cells and the effect of licochalcone A on the parasites *in vivo* may well also be due to intracellular killing of the parasites in the macrophages since it is understood from this example that licochalcone is taken up by the cells.

EXAMPLE 25

Detection of the half live of licochalcone A in mice

Standards. Standards containing 14.68 µg/ml, 4.90 µg/ml, and 0.45 µg/ml plasma were freshly prepared every day. The standards were wrapped in tinfoil to avoid light catalyzed isomerization.

A solution of 2,4-dimethylnitrobenzene (0.20 µl/ml) was used as an internal standard.

Licochalcone A (10 mg/kg mouse ≈ 200 µg/mouse) was injected i.p. into mice. The mice were sacrificed and bleeded after 15, 30, 60, 120, 240 and 480 min. The plasma was obtained by centrifugation. 100 µl of plasma was added to 100 µl of the solution of the internal standard and the mixture left for 30 min. The mixture was centrifuged for 5 min at 10,000 g and 20 µl of the clear supernatant was analyzed by HPLC (Example 23).

Results

Samples	Licochalcone A µg/ml
Control	0
Mouse bleeded after 15 min	1.33
- after 30 min	0.28
- after 60 min	0.14
- after 120 min	below det. limit
- after 240 min	below det. limit
- after 480 min	below det. limit

Beside the peak corresponding to licochalcone A additional peaks were observed. Three peaks were detected by changing the eluent to acetonitrile-2% aqueous acetic acid (43:57), lica-M_1, k' 2.2, lica-M_2, k' 0.69, and lica-M_3 k' 1.3, licochalcone A, k' 8.3. Cleavage of the two fast moving peaks was catalyzed by a β-glucoronidase (*Helix pomatia*, Sigma), which is contaminated with sulfatase. The UV-visible spectra of these compounds were similar to that of licochalcone A. The slow moving metabolite had λ_{max} at much lower wavelength than licochalcone A.

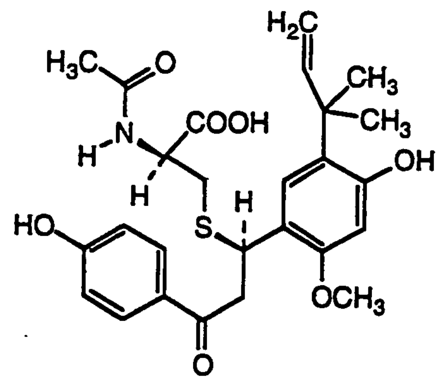
Conclusion

A half live of approximately 18 min is calculated assuming that licochalcone A is metabolized according to a 1-compartment model and eliminated by a first order mechanism. Three metabolites have been detected. The two fast moving metabolites are either glucoronides, sulfates or glucoronides and sulfates. The low wavelength at which λ_{max} of the slow moving metabolite was located indicated that the double bond of the α,β-ketone has been changed or that the aromaticity of the A-ring was lost.

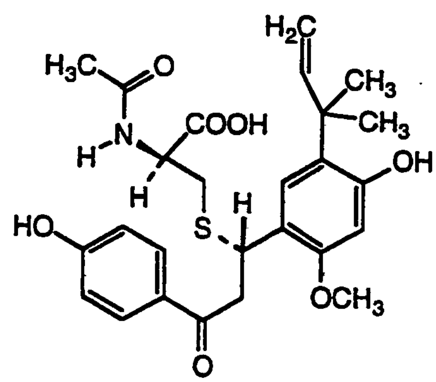
EXAMPLE 26

Reaction between licochalcone A and thiol-containing peptides

Formation of N-acetyl-L-cysteine conjugates of licochalcone A. A solution of 1.5 mg of licochalcone A and 100 mg of N-acetyl-L-cysteine in acetonitrile-0.13 M aqueous potassium phosphate buffer solution pH 7.5 (1:3) was left for 7 days at ambient temperature protected from light. Analysis of the solution by HPLC (Example 23) using acetonitrile-2% aqueous acetic acid (45:55) as an eluent showed decreased amounts of licochalcone A and appearance of two new peaks probably the two conjugates of licochalcone A and N-acetyl-L-cysteine of the below formulae A and B.



A



B

No similar decrease of the concentration of licochalcone A was seen when the chalcone was dissolved in the buffer and left for 7 days without addition of N-acetyl-L-cysteine.

The same two conjugated were formed by reacting licochalcone A with N-acetyl-L-cysteine in a preparative scale:

22 mg (70 µmole) of licochalcone A and 1.4 g (8.5 mmol) of N-acetyl-L-cysteine were dissolved in methanol-water-0.2 M potassium phosphate buffer, pH 8.5 (2:9:9) and the

solution was left for 4 days at room temperature. The solution was concentrated *in vacuo* to half volume and the two conjugates were isolated by HPLC over PLRP-S (3 µm) using acetonitrile-methanol-0.1 M ammonium acetate buffer, pH 4.0 (2:9:9) as an eluent. The appropriate fractions were concentrated *in vacuo* to half the volume and the remaining solvent removed by freeze drying. The procedure of dissolving the residue was dissolved in water and freeze drying was repeated several times in order to remove remaining amounts of ammonium acetate. The two conjugates were isolated in yields of 20 (57%) and 10 mg (28%), respectively.

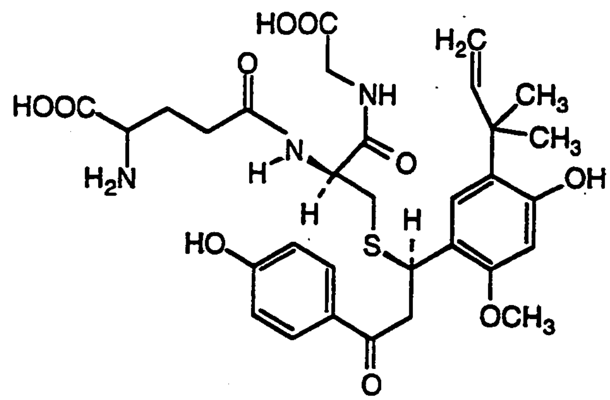
^{1}H NMR data for conjugate I (400 MHz, CD_3OD, δ) 7.79 (AA'-part of an AA'MM'-system, H-2' and H-6'), 7.17 (s, H-2), 6.80 (MM'-part of an AA'MM'-system, H-3' and H-5'), 6.30 (s, H-5), 6.17 (dd, *J* 18 and 10 Hz, CH=), 4.88 (signal partly hidden by CD_3OH signal, CH_2=), 4.82 (dd, *J* 8.4 and 6.8 Hz, H-β), 4.40 (dd, *J* 7.6 and 4.3 Hz, H-α (cysteine)), 3.71 (s, CH_3O), 3.5-3.3 (signal partly hidden by CHD_2O, AB-part of an ABX-system, 2H-α), 2.96 (dd, *J* 13.2 and 4.3 Hz, H-β (cysteine)), 2.82 (dd, *J* 13.2 and 7.6 Hz, H-β (cysteine)), 1.92 (s, CH_3CO), 1.38 (s, $(CH_3)_2C$=).

^{13}C NMR data for conjugate I (50 MHz, CD_3OD, δ) 196.0, 176.6, 172.6, 163.8, 157.4, 156.9, 149.5, 132.1., 130.2, 127.9, 127.3, 120.5, 116.2, 110.4, 101.1, 56.1, 55.8, 45.5, 41.2, 41.1, 35.4, 27.6, 22.8.

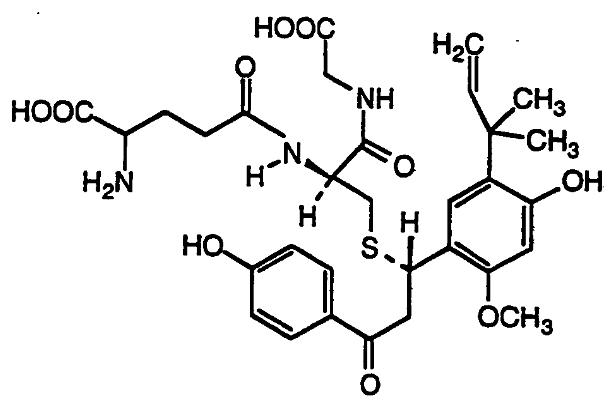
^{1}H NMR data for conjugate II (400 MHz, CD_3OD, δ) 7.82 (AA'-part of an AA'MM'-system, H-2' and H-6'), 7.21 (s, H-2), 6.80 (MM'-part of an AA'MM'-system, H-3' and H-5'), 6.30 (s, H-5), 6.18 (dd, *J* 18 and 10 Hz, CH=), 4.9 (signal partly hidden by CD_3OH signal, CH_2=), 4.9 (signal partly hidden by CHD_2O, AB-part of an ABX-system, 2H-β), 4.48 (t, *J* 5.7 Hz, H-α (cysteine)), 3.69 (s, CH_3O), 3.5-3.3 (signal partly hidden by CHD_2O, AB-part on an ABX-system, 2H-α), 2.81 (d, *J* 5.8 Hz, H-β (cysteine)), 2.00 (s, CH_3CO), 1.39 (s, $(CH_3)_2C$=).

^{13}C NMR data for conjugate II (50 MHz, CD_3OD, δ) 197.3, 174.7, 170.7, 161.8, 155.6, 154.9, 147.6, 130.2, 128.3, 126.3, 125.3, 118.7, 114.5, 108.4, 99.0, 54.0, 53.6, 43.7, 39.2, 39.0, 33.1, 25.7, 20.9.

Formation of glutathione conjugates of licochalcone A. A solution of 1.5 mg of licochalcone A and 100 mg of glutathione in acetonitrile-0.13 M aqueous potassium phosphate buffer solution pH 7.5 (1:3) was left for 7 days at ambient temperature protected from light. Analysis of the solution by HPLC (Example 23) using acetonitrile-2% aqueous acetic acid (45:55) as an eluent showed decreased amounts of licochalcone A and appearance of two new peaks probably the two conjugates of licochalcone A and glutathione of the below formulae C and D.



C

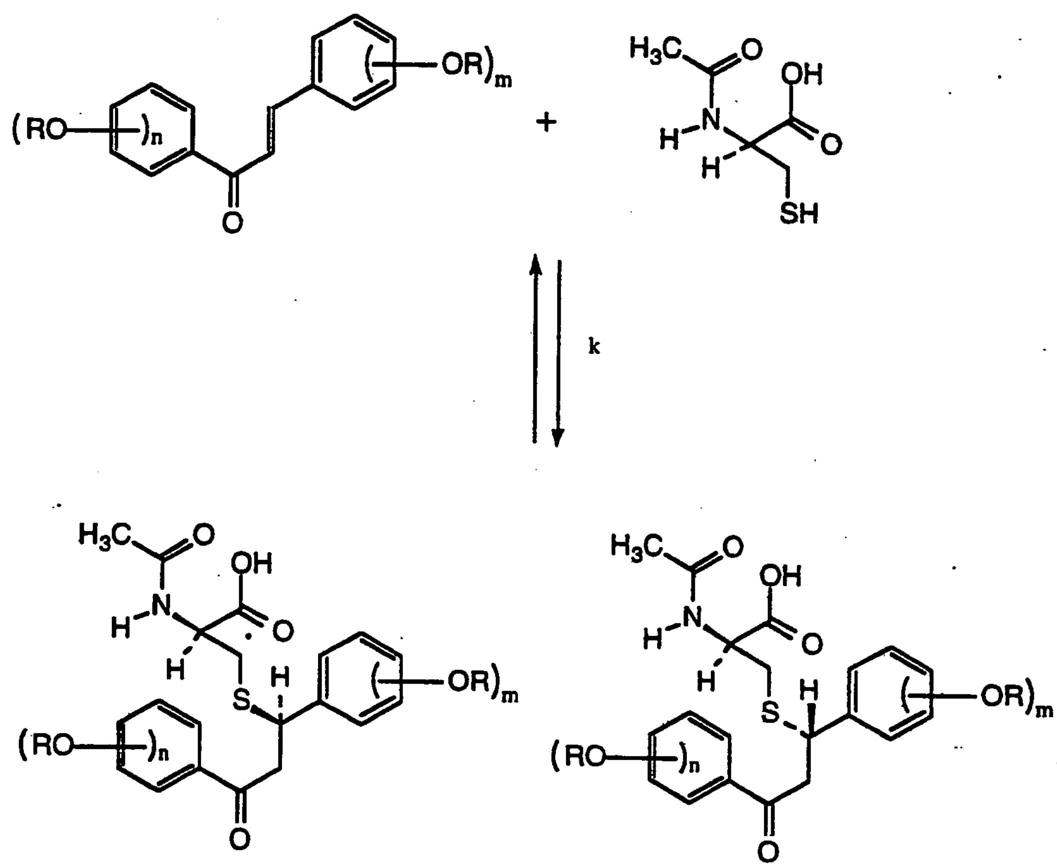


D

No similar decrease was seen when only licochalcone A was dissolved in the buffer and left for 7 days.

EXAMPLE 27

Estimation of the rate of the reaction between N-acetyl-L-cysteine and chalcones



150 µg of the chalcone in question and 10 mg (0.06 mmol) of N-acetyl-L-cysteine were dissolved in 2.5 ml of methanol-water-potassium phosphate buffer (40:10:50 v/v, pH 7.5), and the solution was left at 30°C.

The decline of the chalcone concentration was followed by HPLC. The following experimental setups were used:

1. Column Spherisorb ODS-2 (120 x 4.6 m, 5 µm), eluent acetonitrile-aqueous acetic acid (2%) in ratios 43:57 or 50:50, flow rate 1.5 ml/min, detection at 254 and 360 nm.

The polar eluent was used for the following chalcones: licochalcone A, chalcone, 2,4-dimetoxychalcone and 4,4'-dihydroxychalcone. The apolar eluent was used for the following chalcones: 3,4,5-trimethoxy-4'-(3-methylbut-2-enyloxy)chalcone, 2,4-dimethoxy-4'-(3-methylbut-2-enyloxy)chalcone and 2,4,6-trimethoxy-4'-(3-methylbut-2-enyloxy)-chalcone.

2. Column Polygosil Si 60 (120 x 4.6 mm, 5 µm), eluent methanol-water-0.2 M potassium phosphate buffer (65:30:5, pH 7.5) added cetyltrimethylammonium bromide to a final concentration of 2.5 mM, column temperature 45°C, flow rate 1.0 ml/min, detection at 254 nm. This system was used i the case of 4'-methoxychalcone.

Results

The rate of decline of the chalcone concentration was followed by HPLC. The rate constant was estimated using Grafit and assuming that the reaction followed first order kinetic; i.e.:

$$[c] = [c_0]\exp(-kt)$$

where [c] is the concentration of the chalcone in question at time t, $[c_0]$ is the concentration of the chalcone in question at time zero, k is the rate constant, and t the time.

In all cases, very good fits between the observed concentrations and the concentrations calculated using the estimated rate constants were obtained.

Table 27.1. Estimated rate constants.

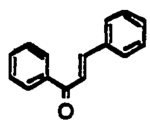
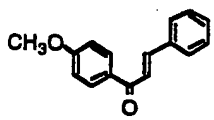
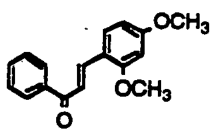
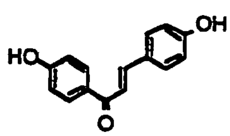
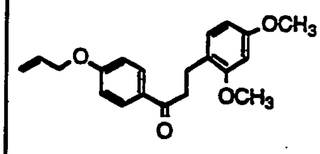
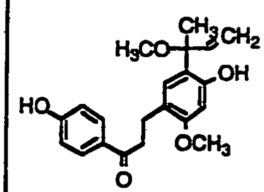
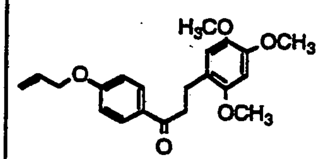
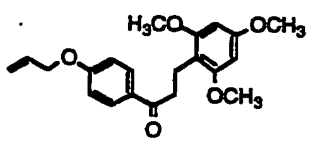
Formula	Number of oxygens	k (min^{-1})
	0	0.034
	1	0.016
	2	0.0033
	2	0.0020

Table 27.1 continued.

Formula	Number of oxygens	k (min^{-1})
	3	0.0011
	3	0.00058
	4	0.0175
	4	0.0006

Conclusion

Introduction of oxygen functions in the 2-position, the4-position or the 2- and the 4-positions, or in the 4'-position appears to decrease the reaction rate. In contrast, comparison of the rate constants of 3,4,5-trimethoxy-4'-(3-methylbut-2-enyloxy)chalcone and 2,4,6-trimethoxy-4'-(3-methylbut-2-enyloxy)chalcone indicates that introduction of oxygen functions in the 3- and 5-position increases the electron density at the double bond and consequently reduces the reactivity toward nucleophilic reagents, whereas the inductive effects of oxygen in the 3- or 5-position will decrease the electron density at the double bond. Analogously, the Hammet σ_p constant for methoxy is -0.27 but σ_m is 0.12.

EXAMPLE 28

Anticoccidial activity of licochalcone A, 2,5-dimethoxy-4'-allyloxychalcone and 2,4-dimethoxychalcone in chickens

The experiment was carried out in collaboration with Korn og Foderstof Kompagniet (KFK) at KFK's Experimental Station (Forsøgsgård, Sdr. Forumvej 18, DK-6715 Esbjerg, Denmark).

Test compounds. 1) Licochalcone A
2) 2,5-Dimethoxy-4'-allyloxychalcone (2,5m4'ac)
3) 2,4-Dimethoxychalcone (2,4mc).

The three compounds were mixed manually with chicken feed one week before use. 2.6 g licochalcone A, 20.6 g 2,4-dimethoxy-4'-chalcone and 20.1 g 2,4-dimethoxychalcone were each mixed separately with 1 kg rye flour. Each of the 1 kg mixtures was then mixed with ten kg chicken feed. Appropriate concentrations of each compound were obtained by adding more chicken feed. The prepared feed as well as a standard feed used, containing 70 ppm salinomycin which is a known coccidiostatic agent, were stored at a temperature between 10°C and 15°C prior to use.

Parasite strain. *Eimeria tenella* sporulated oocysts were obtained from the Agricultural and Food Council Institute for Animal Health, Compton Laboratory, Compton Nr. Newbury, Berkshire RG 16 ONN, England. Permission to import *Coccidia* into Denmark from England has been obtained from the Danish Veterinary authorities, Ministry of Agriculture (Veterinærdirektoratet, Landbrugsministeriet). The oocysts were washed and resuspended in 30 ml saline to give a concentration of $15 \times 10^6/30$ ml. A volume of 0.1 ml (50,000 oocysts) was given to each chicken by a Tuberculin syringe in the crop.

Anticoccidial testing. The experimental set-up consisted of 6 groups of 14-days old chickens. During the first 14 days of life, all the chickens received chicken feed containing no coccidiostatic agents. The first 5 groups were given 50,000 *E. tenella* oocysts per chicken by oral administration on day 14. Feeding the chickens with the feed preparations described above started one day before infection with the parasite (day 13). The treatment continued for 14 days according to the set-up shown in Table 28.1.

Table 28.1. Experimental set-up for testing anticoccidial activity of licochalcone A and two of its analogues.

Groups	Number	Infection with *E. tenella*	Treatment
1	35	Yes	Standard KFK feed containing salinomycin
2	35	Yes	Compound 2,5m4'ac (30 mg/kg chicken body weight/day)
3	35	Yes	Compound 2,4mc (30 mg/kg chicken body weight/day)
4	20	Yes	Licochalcone A (10 mg/kg chicken body weight/day)
5	35	Yes	None
6	35	Yes	None

The following parameters were examined and the samples were obtained:

1. Before infection with oocysts approximately 5 ml of pooled blood sample were taken from 2-5 chickens. Serum from these samples was prepared and stored at a temperature of -20°C.

2. All the chickens were weighed once a week as a standard procedure.

3. Mortality of the chickens was observed and recorded on a daily basis.

4. At the end of the experiment (14 days treatment, 28 days old chickens), the chickens were slaughtered and a necropsy was performed for identification of gross pathology. Histopatholology was registered in standard HE sections of 10-15 mm of one cecal sac, one transverse and one longitudinal. The sections from each chicken were examined. The pathology was registered according to J. Johnson and W. M. Reid, Anticoccidial drugs: Lesion scoring techniques in battery and floor pen experiments with chickens. *Experimental Parasitology* 28 (1970), 30-36.

5. Parasite load in the intestine or number per smear may be determined at the end of the experiment. The number of oocysts in 10 viewfields may be counted at 100 x enlargement and the average of 10 fields were used. Oocysts index may calculated as:

$$\frac{\text{Oocysts in infected animal/field} \times 100}{\text{Oocysts in control animal/field}}$$

and may be recorded as follows:

0	= no oocysts
+	= 1 oocysts/field
++	= 1-10 oocysts/field
+++	= >10 oocysts/field

6. Blood samples for measurement of concentrations of these compounds were obtained from groups 2, 3, and 4 as follows:

1. Prior to treatment with the compounds.
2. Seven days after initiation of treatment.
3. At the end of the experiment before slaughtering the chickens.

Table 28.2. Effect of licochalcone A and two of its analogues on weight gain of chickens. The groups are as shown in Table 28.1. The weights are given in grams per chicken.

Group	Weight	Before treatment	After initiation of treatment	
	7 days old	Weight gain in 7 days	Weight gain in 7 days	Weight gain in 14 days
1	148	215	425	900[a]
2	138	207	366	804
3	138	200	380	803
4	135	201	414	864[b]
5	138	200	367	803
6	130	204	404	866

[a] Difference between groups 1 (standard feed) and 5 (control infected) after treatment for 14 days is 12%.

[b] Difference between groups 4 (licochalcone A) and 5 (control infected) after treatment for 14 days is 7.6%.

Normal variation in such experiments is ±2%.

Table 28.3. Effect of licochalcone A and two of its analogues on feed consumption and mortality of chickens. The groups are as shown in Table 28.1.

Group	Before treatment	After initiation of treatment		Mortality
	after 14 days	after 7 days	after 14 days	
1	1.10	1.30	1.44	0
2	1.12	1.31	1.54	2
3	1.13	1.30	1.48	1
4	1.13	1.26	1.31	0
5	1.15	1.36	1.48	1
6	1.11	1.32	1.46	0

Table 28.4. Effect of licochalcone A and two of its analogues on gross lesions induced by *E. tenella* infection. The pathological scores are according to Johnson and Reid and are given as number of chickens with + to ++++ gross pathology/total number in each group. Total percentages with pathological changes are given in the last column.

Group	Pathological scores			% chicken
	Total	Score	Numbers	
1	0/20	0	20	0%
		+	0	
		++	0	
		+++	0	
2	13/20	0	7	65%
		+	8	
		++	0	
		+++	2	
		++++	3	
3	17/20	0	3	85%
		+	12	
		++	2	
		+++	2	
		++++	1	
4	7/20	0	13	35%
		+	6	
		++	0	
		+++	1	
		++++	0	
5	35/40	0	5	87.5%
		+	24	
		++	5	
		+++	4	
		++++	2	
6	0/40	0	40	0%
		+	0	
		++	0	
		+++	0	
		++++	0	

0 = normal
+ = few hemorrhages (punctuate)
++ = blood in lumen, mucosal lesions, thickened walls
+++ = blood in lumen (coagulated, clumps), detached epithelium
++++ = diffuse bleeding, obstructed cecum, big masses mixed with lots of oocysts

Conclusions

The results from this experiment clearly indicate that licochalcone A is able to control *E. tenella* infection in chickens. This is documented by the following:

1. No mortality was observed in the group receiving licochalcone A (Table 28.3).

2. A 7.6% increase in weight gain in chickens receiving licochalcone A as compared to the infected group not receiving any cocciostatic treatment (Table 28.2). The normal variation in such experiments is ±2%.

3. Feed consumption, measured as the amount of feed consumed per kg chicken weight gain in the group receiving licochalcone A was the lowest among all the 6 groups both on 7 days of treatment and on 14 days of treatment (Table 28.3). The feed consumption of the group receiving licochalcone A was even lower than the group receiving standard chicken feed containing salinomycin which is a know cocci-diostatic agent. This indicates that licochalcone A might have a growth promoting effect or another form of nutritional value.

4. The percentages of chickens showing pathological signs were much lower in the group receiving licochalcone A than in the infected control group (Table 28.4).

The chickens receiving licochalcone A did not perform the same way as those receiving standard chicken feed containing salinomycin. However, when comparing the licochalcone A group with the group receiving standard feed, it should be noted that this group received standard feed which besides a coccidiostatic agent also contains larger amounts of nutrients, vitamin, and growth promoting factors.

In the above experiment, licochalcone A did not show a complete protection against *E. tenella* infection. This is probably due to the dosage of licochalcone A used in the experiment. It should also be mentioned that the experimental infection is a much stronger form of infection than the infection which will normally be encountered in practice.

REFERENCES

Ann (WHO), Wkly Epidem Rec. 1990, vol. 65, 189-196

Ashford, R. W.: "The Leishmaniases", Clinics in Tropical Medicine and Communicable Diseases, eds. H. M. Gilles, vol. 1, No. 3, W. B. Saunders Comp. Ltd., London, 1986 513-533.

Berenguer, E., Moreno S., Cercenado E., Quiros JCLBd, Fuente AGdl, Bouza E.: "Visceral leishmaniasis in patients infected with human immunodeficiency virus (HIV)", Ann. Intern. Med. 1989, vol. 111, 129-132.

Berman, J.D., Dwyer, D.M., and Wyler, D.J.: "Multiplication of Leishmania in human macrophages in vitro", Infect. Immun., vol. 26, 1979, 375-379.

Bowden, K. , Duah, C. K., and Ranson, R. J.: *J. Chem. Soc. Perkin Trans.* 2 (1990), 109.

Davis, and Armstrong: *J. Am. Chem. Soc.* 57 (1935), 1583.

Flegg PJ, Brettle RP: "Visceral Leishmaniasis in HIV-Infected Patients", AIDS 4(4), 1990, 366-367.

Geissman, T. A., Clinton, R. O.: *J. Am. Chem. Soc.* 68 (1946), 697-700.

Hatano, T., Kagawa, H., Yasuhara, T., and Okuda, T.: "Two new flavonoids and other constituents in licorice root: Their relative astringency and radical scavenging effects", *Chem. Pharm. Bull.* 36(6) (1988), 2090-2097.

Hébert, G. Ann, Callaway, Carey S., and Ewing, Edwin P.: "Comparison of Legionella pneumophila, L. micdadei, L. bozemanii and L. dumoffii by Transmission Electron Microscopy", *J. Clin. Microbial* 19 (1984), 116-121.

Inoue, B. Inaba, K., Mori, T., Izushi, F., Eto, K., Sakai, R., Ogata, M. and Utsumi, K. *J. Toxicol. Sci.* 7 (1982), 245-254.

Jensen JB, Boland MT, Hayes M., *Exp. Parasitol.* 54 (1982), 416-424.

Johnson, J. and Reid, W. M.,*Experimental Parasitology* 28 (1970), 28-30.

Kenzo Okada, Yukiyoshi Tamura, Masaji Yamamoto, Yoshimoto Inoûe, Ryoji Takagaki, Kunio Takahashi, Sachio Demizu, Kiichiro Kajiyama, Yukio Hiraga and

Takeshi Kinoshita: "Identification of Antimicrobial and Antioxidant constituents from Licorice of Russian and Xinjingang Origin", *Chem. Pharm. Bull.* 37 (1989), 2528-2530.

Khan, S. A., and Krishnamurti, M.: *Indian J. Chem.* 22B (1983), 276.

Kimura, Y., Okuda, H., Okuda, T., and Arichi, S.: "Effects of chalcones isolated from licorice roots on leukotriene biosynthesis in human polymorphonuclear neutrophils", *Phytotherapy Research* 2(3) (1988), 140-145.

Kurosawa, K., annd Higuchi, J.: *Bull. Chem. Soc. Japan* 45 (1972), 1132-1136.

Kurosawa, K.: *Bull. Chem. Soc. Japan* 42 (1969), 1456.

Kyogoku, K., Hatamaya, K., Yokomori, S., Saziki, R., Nakane, S., Sasajima, M., Sawada, M., Ohzeki, M., Tanaka, I.: *Chem. Pharm. Bull.* 27 (1979), 2943.

Lambros C., Vanderberg JP.: "Synchronization of *Plasmodium falciparum* erythrocytic stages in culture", *J. Parasitol.* 65 (1979), 418-420.

Laurushin, V. F., Trusevich, N. D., and Tolmachev, V. N.: *Zh.. Obshch Khim.* 39 (1969), 42-45, *Chem. Abstr.* 70 (1969), 105822t.

Liew F.Y., Millott, S., Parkinson, C., Palmer, R.M.J., and Moncada, S.: "Macrophage killing of Leishmania parasite in vivo is medicated by nitric oxide from L-arcinine", *J. Immun.* 144 (1990), 4794-4797.

Manson-Bahr, C.E.P. and Bell, R.D.: "Manson's Tropical Diseases", Bailliére Tindall, 19 Ed., 1987 B.

Nielsen, A. T. and Houlihahn, W.: *J. Org. React.* 16 (1968), 1.

Pearson, R., A.A. Manian, D. Hall, J.L. Harcus, and E.L. Hewlett: "Antileishmanial activity of chlorpromazine", *Antimicrob. Agents Chemother.* 25 (11984), 571-574.

Ren Jun, Wang Zhengang: "Pharmacological research on the effect of licorice": *J. Trad. Chinese Med.* 3 (1988), 307-309.

Rhen-Seng, X., Kung-Ling, W., Shifa J., Chang-gen, W., Fu-Xiang, J., Xu-yan, X., and Yi-Sheng, G.: *Acta Chem. Sinica* 37 (1979), 289-297.

Ristroph, Joseph D., Hedlund, Kenneth W., and Allen, Richard G.: "Liquid medium for growth of Legionella pneumophila": *J. Clin. Microb.* 11 (1980), 19-21.

Sachio Demizu, Kiichiro Kajiyama, Kunio Takahashi, Yukio Hiraga, Susumu Yamamoto, Yukiyoshi Tamura, Kenzo Okada and Takeshi Kinoshita: *Chem. Pharm. Bull.* 36 (1988), 3474-3479.

Saitoh, T., and Shibata, S.: *Tetrahedron Lett.* 50 (1975), 4461.

Sallai, J., Gabor, M., and Kallay, F.: *Acta Farm. Hung.* 46 (1976), 49-56.

Salmon D., Deloron P., Gaudin C., Malhotra K., Lebras J., Pocidalo JJ.: "Activities of peploxacin and ciprofloxacin against experimental malaria in mice", *Antimicrob. Agents Chemother.* 34 (1990), 2327-2330.

Sheng, L.H.: "Traditional Chinese medicine and immunity", Guangdong Scientific Publishing House, Guangzhou, People's Republic of China, 41-49, 1982

Shriner, R. L., Kurosawa, T.: *J. Am. Chem. Soc.* 52 (1930), 2538.

Swarbrick, J. and Boylan, J. C.: "EWmcyclopedia of Pharmaceutical Technology", Marcel Dekker, Inc., New York, 1988.

Swarbrick, J. and Boylan, J. C.: "Remington's Pharmaceutical Sciences", Marcel Dekker, Inc., New York, 1988.

Teelucksingh, S. et al.: "Liquorice", *The Lancet* 37 (1991), 1549.

Thoda, Y, Sonogashihare, K., and Haghara, N.: *Synthesis* (1977), 777.

Trager, W., Jensen JB.: "Human malaria in continuous cultures", *Science* 193 (1976), 673-675.

UNDP/World Bank/WHO Special Programme for research and Training in Tropical Diseases (TDR), Ninth Programme Report, Tropical Diseases: Progress in international research, 1987-1988, "The leishmaniases", 85-92, WHO, Geneva 1989.

UNDP/World Bank/WHO: "Antimonial: Large-scale failure in leishmaniasis "alarming"", TDR News, vol. 34, December 1990, 1, 7.

Volovich, A. M., Tolmachev, V. N., and Larushin, V. F.: *Visn. Kharkiv Univ. Khim.*

73 (1971), 85-88, *Chem. Abstr.* 78 (1973), 57241u.

Walton, C. B.: "Leishmaniasis", Tropical Medicine and Parasitology, eds. R. Goldsmith & D. Heyneman, Printece-Hall Int. Editions, 1989, 276-302.

Wattanasin, S. and Murphy, S.: *Synthesis* (1980), 647.

WHO: "Global Estimates for Health Situation Assessment and projections-1990", WHO/HST/90.2, 1990, 18-33, A.

CLAIMS

1. The use of an aromatic compound, or a prodrug thereof, which aromatic compound contains an alkylating site and which aromatic compound is capable of alkylating the thiol group in N-acetyl-L-cysteine at physiological pH, for the preparation of a pharmaceutical composition or a medicated feed, food or drinking water for the treatment or prophylaxis of a disease caused by a microorganism or a parasite in an animal, including a vertebrate, such as a bird, a fish or a mammal, including a human,

the microorganism or parasite being selected from

parasitic protozoa, in particular tissue and blood protozoa such as *Leishmania, Trypanosoma, Toxoplasma, Plasmodium, Pneumocystis, Babesia* and *Theileria;* intestinal protozoan flagellates such as *Trichomonas* and *Giardia;* intestinal protozoan *Coccidia* such as *Eimeria, Isospora, Cryptosporidium; Cappilaria, Microsporidium, Sarcocystis, Trichodina, Trichodinella, Dacthylogurus, Pseudodactylogurus, Acantocephalus, Ichthyophtherius, Botrecephalus;* and intracellular bacteria, in particular *Mycobacterium, Legionella* species, *Listeria,* and *Salmonella.*

2. The use according to claim 1, wherein the aromatic compound, in a concentration in which it causes less than 50% reduction of the thymidine uptake by human lymphocytes in the Lymphocyte Proliferation Assay using phytohemagglutinin (PHA), meets at least one of the following criteria:

a) the aromatic compound is capable of inhibiting *in vitro* the growth or multiplication of *Leishmania major* promastigotes by at least 80%, as determined by uptake of tritiated thymidine,

b) the aromatic compound is capable of inhibiting *in vitro* the growth or multiplication of *Plasmodium falciparum* by at least 80%, as determined by uptake of tritiated hypoxanthine,

c) the aromatic compound is capable of inhibiting *in vitro* the growth or multiplication of *Eimeria tenella* in chicken fibroblast cell cultures by at least 70% ,as determined by counting the parasites,

d) the aromatic compound is capable of inhibiting *in vitro* the growth or multiplication of *Mycobacterium tuberculosis* or *Legionella pneumophila* by at least 50%, as determined by colony counts.

3. The use according to claim 2, wherein the aromatic compound meets all of the criteria

a) to d).

4. The use according to claim 2, wherein the aromatic compound, in a concentration in which it causes less than 40% reduction of the thymidine uptake by human lymphocytes in the Lymphocyte Proliferation Assay using PHA, meets at least one of the criteria a) to d).

5. The use according to claim 4, wherein the aromatic compound meets all of the criteria a) to d).

6. The use according to claim 2, wherein the aromatic compound, in a concentration in which it causes less than 20% reduction of the thymidine uptake by human lymphocytes in the Lymphocyte Proliferation Assay using PHA, meets at least one of the criteria a) to d).

7. The use according to claim 6, wherein the aromatic compound meets all of the criteria a) to d).

8. The use according to claim 1, wherein the pharmaceutical composition is a composition for the treatment or prophylaxis of diseases caused by *Leishmania* in humans or dogs, and the aromatic compound is capable of inhibiting *in vitro* the growth of *Leishmania major* promastigotes by at least 80%, as determined by uptake of tritiated thymidine, in a concentration of the compound in which it causes less than 50% reduction of the thymidine uptake by human lymphocytes in the Lymphocyte Proliferation Assay using PHA.

9. The use according to claim 8, wherein the aromatic compound is capable of inhibiting *in vitro* the growth of *Leishmania major* promastigotes by at least 80%, as determined by uptake of tritiated thymidine, in a concentration of the compound in which it causes less than 40% reduction, preferably less than 20% reduction, of the thymidine uptake by human lymphocytes in the Lymphocyte Proliferation Assay using PHA.

10. The use according to claim 1, wherein the pharmaceutical composition is a composition for the treatment or prophylaxis of diseases caused by *Leishmania* in humans or dogs, and the aromatic compound, or the prodrug, when administered intraperitoneally in the *in vivo* test described in Example 8 herein in a dose of up to 20 mg per kg body weight once daily for 40 days to female BALB/c mice which have been infected with *L. major* (10^7/mouse), the administration being initiated one week after infection, is capable of preventing increase in lesion size by at least 60%, preferably at least 80%, more preferably at least 90%.

11. The use according to claim 10, wherein the aromatic compound, or the prodrug, when

administered intraperitoneally in the *in vivo* test described in Example 8 herein in a dose of up to 10 mg per kg body weight once daily for 40 days to female BALB/c mice which have been infected with *L. major* (10^7/mouse), the administration being initiated one week after infection, is capable of preventing increase in lesion size by at least 60%, preferably at least 80%, more preferably at least 90%.

12. The use according to claim 1, wherein the pharmaceutical composition is a composition for the treatment or prophylaxis of diseases caused by *Leishmania* in humans or dogs, and the aromatic compound, or the prodrug, when administered intraperitoneally in the *in vivo* test described in Example 9 herein in a dose of up to 20 mg per kg body weight two times daily for 7 days to male Syrian golden hamsters which have been infected with *L. donovani* promastigotes (2 x 10^7/hamster), the administration being initiated one day after infection, is capable of reducing the parasite load in the liver of the hamsters by at least 60%, preferably by at least 80%, and more preferably by at least 90%.

13. The use according to claim 12, wherein the aromatic compound when administered intraperitoneally in the *in vivo* test described in Example 9 herein in a dose of up to 10 mg per kg body weight two times daily for 7 days to male Syrian golden hamsters which have been infected with *L. donovani* promastigotes (2 x 10^7/hamster), the administration being initiated one day after infection, is capable of reducing the parasite load in the liver of the hamsters by at least 60%, preferably by at least 80%, and more preferably by at least 90%.

14. The use according to claim 1, wherein the pharmaceutical composition is a composition for the treatment or prophylaxis of malaria caused by *Plasmodium spp.* in humans, and the aromatic compound is capable of inhibiting *in vitro* the growth of *Plasmodium falciparum* by at least 80%, as measured by uptake of tritiated hypoxanthine, in a concentration of the compound in which it causes less than 50% reduction of the thymidine uptake by human lymphocytes, as measured by the Lymphocyte Proliferation Assay using PHA.

15. The use according to claim 14, wherein the aromatic compound is capable of inhibiting *in vitro* the growth of *Plasmodium falciparum* by at least 80% in a concentration of the compound in which it causes less than 40% reduction, preferably less than 20% reduction, of the thymidine uptake by human lymphocytes, as measured by the Lymphocyte Proliferation Assay using PHA.

16. The use according to claim 1, wherein the pharmaceutical composition is a composition for the treatment or prophylaxis of diseases caused by *Plasmodium spp.* in humans, and the aromatic compound, when administered intraperitoneally in the *in vivo* test described in Example 16 herein in a dose of up to 20 mg per kg body weight two

times daily for 6 days to female BALB/c mice which have been infected with malaria *P. yoelii* (2×10^5/mouse), the administration being initiated one day after infection, is able to prevent increase in the parasitemia during the administration period.

17. The use according to claim 16, wherein the aromatic compound, or the prodrug, when administered intraperitoneally in the *in vivo* test described in Example 16 herein in a dose of up to 20 mg per kg body weight two times daily for 10 days to 8 weeks old female BALB/c mice which have been infected with malaria *P. yoelii* (2×10^5/mouse), the administration being initiated one day after infection, is capable of clearing the parasite from the mice within at the most 23 days.

18. The use according to claim 17, wherein the aromatic compound, or the prodrug, when administered intraperitoneally in the *in vivo* test described in Example 16 herein in a dose of up to 20 mg per kg body weight two times daily for 8 days to 8 weeks old female BALB/c mice which have been infected with malaria *P. yoelii* strain YM (1×10^6/mouse), the administration being initiated one day after infection, is capable of clearing the parasite from the mice within at the most 21 days, preferably within at the most 17 days.

19. The use according to claim 18, wherein the aromatic compound, or the prodrug, when administered intraperitoneally in the *in vivo* test described in Example 16 herein in a dose of 5 mg per kg body weight two times daily for 10 days to 8 weeks old female BALB/c mice which have been infected with malaria *P. yoelii* (2×10^5/mouse), the administration being initiated one day after infection, is capable of clearing the parasite from the mice within at the most 23 days.

20. The use according to claim 19, wherein the aromatic compound, or the prodrug, when administered intraperitoneally in the *in vivo* test described in Example 16 herein in a dose of 5 mg per kg body weight two times daily for 8 days to 8 weeks old female BALB/c mice which have been infected with malaria *P. yoelii* strain YM (1×10^6/mouse), the administration being initiated one day after infection, is capable of clearing the parasite from the mice within at the most 21 days, preferably within at the most 17 days.

21. The use according to claim 1 of an aromatic compound, or a prodrug thereof, which aromatic compound contains an alkylating site and which aromatic compound is capable of alkylating the thiol group in N-acetyl-L-cysteine at physiological pH, for the preparation of a pharmaceutical composition or a medicated feed or drinking water for the treatment or propylaxis of diseases caused by *Coccidia* in poultry such as chickens or turkeys, wherein the aromatic compound, or the prodrug, when administered to chickens with the feed in a concentration of up to 400 ppm for at most 28 days in the *in vivo* test described in Example 28 herein, is capable of controlling infection by *Eimeria tenella* in at least 60% of the chickens and preventing pathological alterations in at least 50% of the

chickens.

22. The use according to claim 21, wherein the aromatic compound, when administered to chickens with the feed in a concentration of up to 120 ppm for at most 28 days in the *in vivo* test described in Example 28 herein, is capable of controlling infection by *Eimeria tenella* in at least 60% of the chickens and preventing pathological alterations in at least 65% of the chickens.

23. The use according to claim 1, wherein the pharmaceutical composition is a composition for the treatment or prophylaxis of diseases caused by intracellular bacteria such as *Mycobacteria* in humans or animals such as cattle, and the aromatic compound is one which is capable of inhibiting the growth and multiplication of *Mycobacteria tuberculosis in vitro* in the test described in Example 17 herein at a mean MIC of 10 µg per ml, and, in the same concentration, causes less than 50% reduction of the thymidine uptake of human lymphocytes as measured by The Lympocyte Proliferation Assay.

24. The use according to claim 1, wherein the pharmaceutical composition is a composition for the treatment or prophylaxis of diseases caused by intracellular bacteria such as *Legionella* in humans, and the aromatic compound is one which is capable of inhibiting the growth and multiplication of *Legionella pneumophila in vitro* in the test described in Example 17 herein at a mean MIC of 10 µg per ml, and, in the same concentration, causes less than 50% reduction of the thymidine uptake of human lymphocytes as measured by The Lympocyte Proliferation Assay.

25. The use according to any of the preceding claims, in which the aromatic compound contains an aromatic ring attached to the alkylating site.

26. The use according to any of claims 1-25, in which the compound has electron-donating groups attached to an aromatic ring.

27. The use according to claim 25, wherein the alkylating site is a double or triple bond conjugated with a carbonyl group which carbonyl group optionally is further conjugated with an aromatic ring such as a phenyl group.

28. The use according to any of claims 25, wherein the aromatic ring attached to the alkylating site contains at least one electron donating group such as an oxygen, nitrogen or sulphur function.

29. The use according to claim 26, wherein the electron donating group(s) is/are attached to the aromatic ring in a position next to and/or most remote relative to the position through which the aromatic ring is attached to the alkylating site.

30. The use according to any of claims 1-7, wherein the disease is human leishmaniasis caused by *Leishmania donovani*, *L. infantum*, *L. aethiopica*, *L. major*, *L. tropica*, *L. mexicana complex*, or *L. braziliensis complex* or human malaria caused by *Plasmodium falciparum*, *P. ovale*, *P. vivax*, or *P. malariae*.

31. The use according to any of claims 1-7, wherein the disease is a parasitic disease in livestock, such as *Babesia* in cattle, or a parasitic disease in birds, such as a disease caused by *Coccidia* such as *Eimeria tenella* in poultry such as chicken or turkey, or a parasitic disease in fish, such as *Pseudodactylogurus* or *Trichodina*.

32. The use according to any of the preceding claims, wherein the aromatic compound is a bis-aromatic α,β-unsaturated ketone of the general formula I

$$X_m\text{-}Ar^1\text{-}CO\text{-}W\text{-}Ar^2\text{-}Y_n \qquad \mathrm{I}$$

wherein

W is either -CR=CR- or -C≡C-, wherein each R independently of the other R designates hydrogen, C_{1-3} alkyl, or halogen,

Ar^1 and Ar^2 are the same or different and each designate an aromate selected from phenyl and 5- or 6-membered unsaturated heterocyclic rings containing one, two or three hetero-atoms selected from oxygen, sulfur, and nitrogen, such as furanyl, thiophenyl, pyrrolyl, imidazolyl, isoxazolyl, oxazolyl, thiazolyl, pyrazolyl, pyridinyl, or pyrimidinyl, which aromate may be substituted with one or more substituents selected from

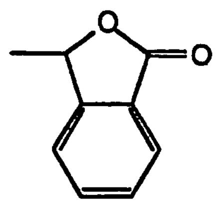
halogen; nitro; nitroso; and C_{1-12}, preferably C_{1-6}, straight or branched aliphatic hydrocarbyl which may be saturated or may contain one or more unsaturated bonds selected from double bonds and triple bonds, which hydrocarbyl may be substituted with one or more substituents selected from hydroxy, halogen, amino, and amino which is optionally alkylated with one or two C_{1-6} alkyl groups;

Y and X are the same or different and each designate a group AR_H or a group AZ, wherein A is -O-, -S-, -NH-, or -N(C_{1-6} alkyl)-, R_H designates C_{1-6} straight or branched aliphatic hydrocarbyl which may be saturated or may contain one or more unsaturated bonds selected from double bonds and triple bonds, and Z designates H or (when the compound is a prodrug) a masking group which is readily decomposed under conditions prevailing in the animal body to liberate a group AH, in which A is as defined abov; m designates 0, 1 or 2, and n designates 0, 1, 2 or 3, whereby, when m is 2, then the two groups X are the same or different, and when n is 2 or 3, then the two or three groups Y

are the same or different, with the proviso that n and m are not both 0.

33. The use according to claim 32, wherein Z, when designating a masking group, is selected from the below groups (A)-(E)

-CO-R′	(A)
$-CON(CH_3)_2$	(B)
-CR*R**-O-R″	(C)
-CR*R**-O-CO-R‴	(D)



(E)

wherein R* and R** each independently designate hydrogen or C_{1-3} alkyl, R′, R″ and R‴ each designate C_{1-6} alkyl or is an aromate Ar^1 or Ar^2 as defined in claim 32.

34. The use according to claims 32 and 33, wherein Ar^1, or Ar^2 or both independently are phenyl or an aromatic 5- or 6-menbered heterocyclic ring containing one, two or three heteroatoms selected from oxygen, sulphur and nitrogen, n is 0, 1, 2, or 3, m is 0, 1 or 2, at least one of the groups X is in a position in Ar^1 most remote relative to and/or next to the position through which Ar^1 is bound to the carbonyl group, and at least one of the groups Y is in a position in Ar^2 most remote relative to and/or next to the position through which Ar^2 is bound to W.

35. The use according to claim 32, in which A is O.

36. The use according to claim 33, in which Z is pivaloyl, pivaloyloxymethyl or N,N-dimethylcarbamoyl.

37. The use according to any of claims 32-36, in which the compound of formula I is a compound of formula II

$$X_m\text{-Ph-C(O)-CH=CH-Ph-}Y_n \qquad \text{II}$$

wherein Ph designates phenyl, and X_m and Y_n are as defined in claim 32, and each phenyl group may be substituted with one or more substituents selected from halogen; nitro; nitroso; and C_{1-12}, preferably C_{1-6}, straight or branched aliphatic hydrocarbyl which may

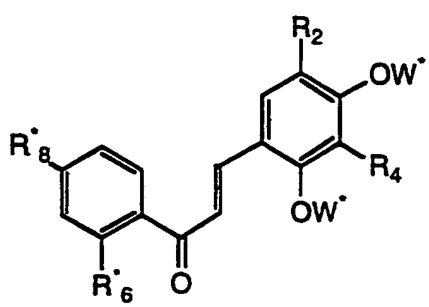
be saturated or may contain one or more unsaturated bonds selected from double bonds and triple bonds, which hydrocarbyl may be substituted with one or more substituents selected from hydroxy, halogen, amino, and amino which is optionally alkylated with one or two C_{1-6} alkyl groups.

38. The use according to claim 37, in which X and/or Y is OH or a group OR_H, in which R_H is as defined in claim 32, or OZ^*, in which Z^* is a masking group which is readily decomposed under conditions prevailing in the animal body to liberate the group OH, in particular one of the groups (A)-(E) as defined in claim 33, preferably pivaloyl, pivaloyloxymethyl or N,N-dimethylcarbonyl.

39. The use according to claim 37, wherein the substituent or substituents on the phenyl groups is/are C_{1-12}, preferably C_{1-6}, straight or branched aliphatic hydrocarbyl which may be saturated or may contain one or more unsaturated bonds selected from double bonds and triple bonds, which hydrocarbyl may be substituted with one or more substituents selected from hydroxy, halogen, amino, and amino which is optionally alkylated with one or two C_{1-6} alkyl groups.

40. The use according to claim 39, wherein the substituent or substituents on the phenyl groups is/are methyl, ethyl, propyl, isopropyl, tert.-butyl, prop-2-enyl, 1,1-dimethylpropyl, 1,1-dimethylprop-2-enyl, 3-methylbutyl, or 3-methylbut-2-enyl.

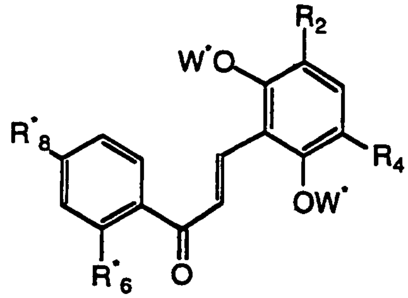
41. The use according to claim 37, wherein the bis-aromatic α,β-unsaturated ketone has the general formula III



III

wherein R_2 and R_4 designate R_H as defined in claim 32 or H, one of R^*_6 and R^*_8 designate OW^* and the other is H, or both R^*_6 and R^*_8 designate H, and W^* designates H, R_H or a group (A)-(E) as defined in claim 33 wherein both R^* and R^{**} designate H.

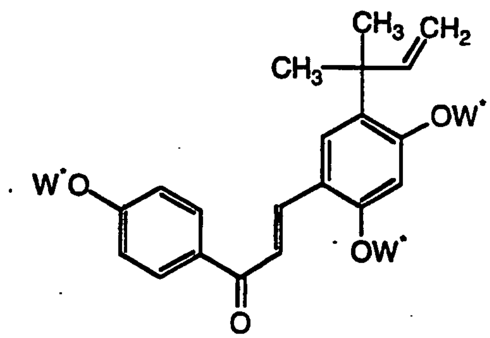
42. The use according to claim 37, wherein the bis-aromatic α,β-unsaturated ketone has the general formula IV



IV

wherein R_2, R_4, R^*_6, R^*_8 and OW^* are as defined in claim 41.

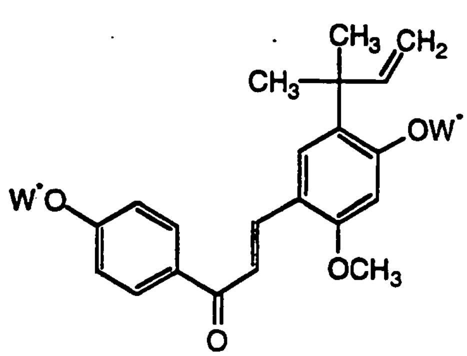
43. The use according to claim 37, in which the bis-aromatic α,β-unsaturated ketone has the general formula V



V

wherein W^* is as defined in claim 41.

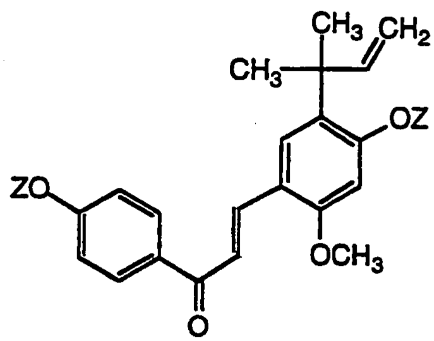
44. The use according to claim 37, in which the bis-aromatic α,β-unstaturated ketone has the general formula VI



VI

wherein W* is as defined in claim 41.

45. The use according to claim 37 in which the bis-aromatic α,β-unsaturated ketone has the general formula VII

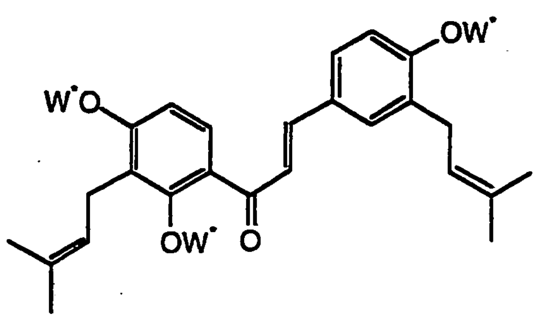


VII

wherein Z is as defined in claim 33.

46. The use according to claim 45, wherein Z designates pivaloyl, pivaloyloxymethyl or N,N-dimethylcarbonyl.

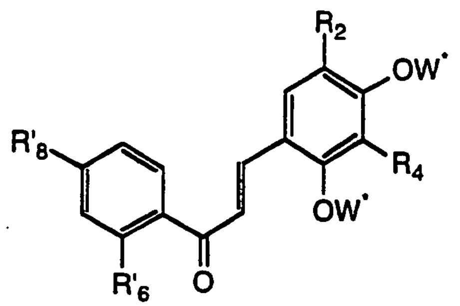
47. The use according to claim 37 in which the bis-aromatic α,β-unsaturated ketone has the general formula VIII



VIII

wherein W* is as defined in claim 41.

48. bis-Aromatic α,β-unsaturated ketones of the general formula IX

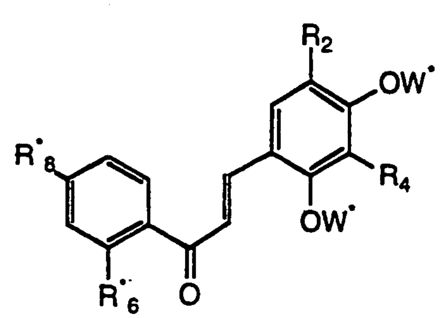


IX

wherein R_2 and R_4 designates R_H or H, where R_H designates C_{1-6} straight or branched aliphatic hydrocarbyl which may be saturated or may contain one or more unsaturated bonds selected from double bonds and triple bonds, one of R'_6 and R'_8 designate $A(W^*)_p$ and the other designates H, or both designate H, A designates S, N or O, whereby, when A designates S or O, then p designates 1, and when A designates N, then p designates 2, with the proviso that when R_2 and R_4 both are H, then at least one W^* designates a masking group Z as defined in claim 32 or 33,

with the exception of licochalcone A, licochalcone C, 3-[4-hydroxy-5-(1,1-dimethylprop-2-enyl)-2-methoxyphenyl]-1-[4-(methoxymethoxy)phenyl]-2-propen-1-one, 3-[4-acetyloxy-5-(1,1-dimethylprop-2-enyl)-2-methoxyphenyl]-1-[4-(methoxymethoxy)phenyl]-2-propen-1-one, 3-[5-(1,1-dimethylprop-2-enyl)-2,4-dimethoxyphenyl]-1-[4-(methoxy)phenyl]-2-propen-1-one, 3-[4-acetyloxy-5-(1,1-dimethylprop-2-enyl)-2-methoxyphenyl]-1-(4-acetyloxyphenyl)-2-prop-1-one, 3-[2-hydroxy-4-methoxy-3-(3-methylbut-2-enyl)phenyl]-1-[4-[(3,7,11-trimethyl-2,6-dodecatri-10-enyl)oxy]phenyl]-2-prop-1-one, and 2,4-dihydroxy-3-methylchalcone.

49. bis-Aromatic α,β-unsaturated ketones according to claim 48 having the general formula X

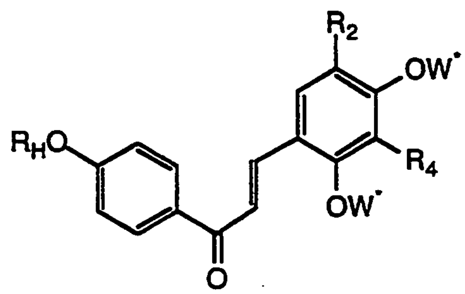


X

wherein R_2 and R_4 are as defined in claim 48, one of R^*_8 and R^*_6 designates OW^*, and the other designates H, or both designate H, and W^* is as defined in claim 48.

50. bis-Aromatic α,β-unsaturated ketones according to claim 49, wherein R_4 designates H.

51. bis-Aromatic α,β-unsaturated ketones according to claim 48 of the general formula XI

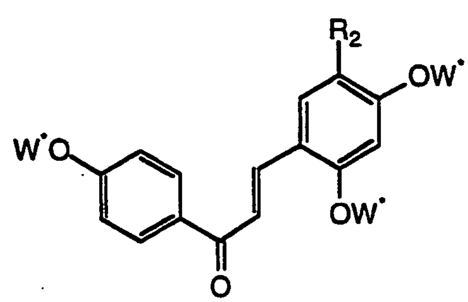


XI

wherein R_2, R_4 and W^* are as defined in claim 48.

52. bis-Aromatic α,β-unsaturated ketones according to claim 48, 49 or 50, in which R_2 and/or R_4 designates methyl, ethyl, propyl, isopropyl, tert.-butyl, prop-2-enyl, 1,1-dimethylpropyl, 1,1-dimethylprop-2-enyl, 3-methylbutyl, or 3-methylbut-2-enyl.

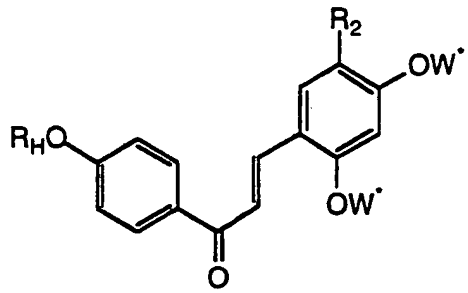
53. bis-Aromatic α,β-unsaturated ketones according to claim 48 of the general formula XII



XII

wherein R_2 and W^* are as defined in claim 48.

54. bis-Aromatic α,β-unsaturated ketones according to claim 53 of the general formula XIII

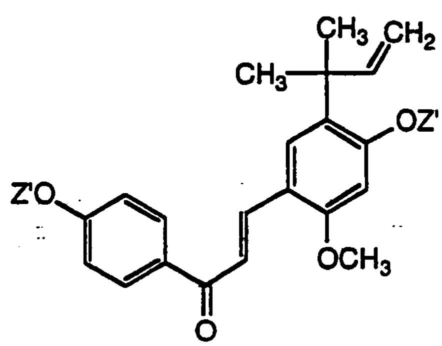


XIII

wherein R_H is as defined in claim 47, and R_2 and $W^{\bullet}$ are as defined in claim 48.

55. bis-Aromatic α,β-unsaturated ketones according to claim 53 or 54, in which R_2 designates methyl, ethyl, propyl, isopropyl, tert.-butyl, prop-2-enyl, 1,1-dimethylpropyl, 1,1-dimethylprop-2-enyl, 3-methylbutyl, or 3-methylbut-2-enyl.

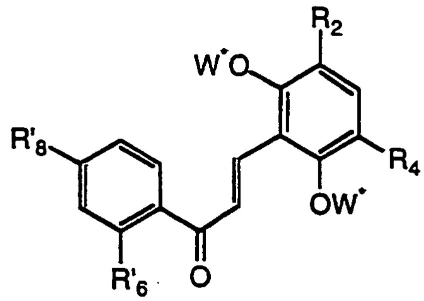
56. bis-Aromatic α,β-unsaturated ketones according to claim 48 of the general formula XIV



XIV

wherein Z' is one of the groups (A)-(E) as defined in claim 33.

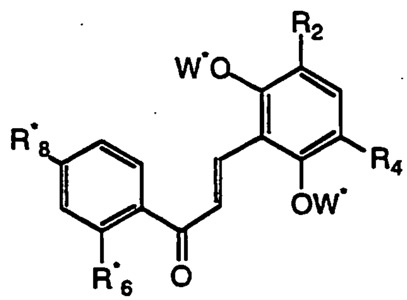
57. bis-Aromatic α,β-unsaturated ketones of the general formula XV



XV

wherein one of R'_6 and R'_8 designates $A(W^*)_p$ and the other designates H, or both designate H, W^* is as defined in claim 41, A designate S, N or O, whereby when A designates S or O then p designates 1, and when A designates N then p designates 2, with the exception of 2,6-methoxychalcone and 2-hydroxy-6-methoxychalcone.

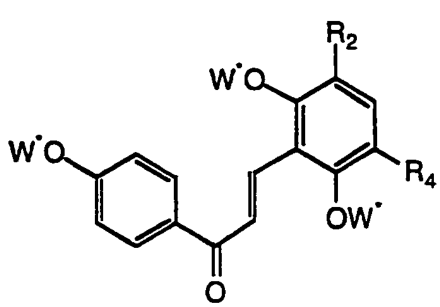
58. bis-Aromatic α,β-unsaturated ketones of the general formula XVI



XVI

wherein R_2, R_4, R^*_8, R^*_6 and W^* are as defined in claim 48.

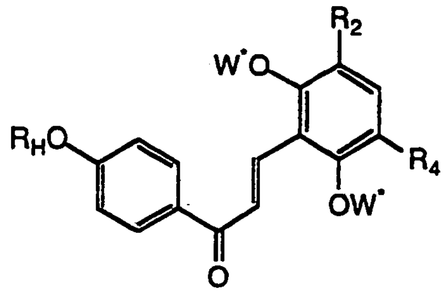
59. bis-Aromatic α,β-unsaturated ketones according to claim 58 of the general formula XVII



XVII

wherein R_2, R_4 and W^* are as defined in claim 48.

60. bis-Aromatic α,β-unsaturated ketones according to claim 58 of the general formula XVIII

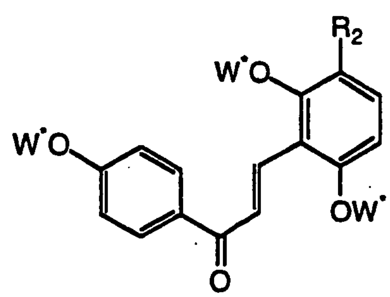


XVIII

wherein R_H isas defined in claim 30, R_2, R_4 and W^* are as defined in claim 48.

61. bis-Aromatic α,β-unsaturated ketones according to any of claims 57-60, in which R_2 and/or R_4 designates methyl, ethyl, propyl, isopropyl, tert.-butyl, prop-2-enyl, 1,1-dimethylpropyl, 1,1-dimethylprop-2-enyl, 3-methylbutyl, or 3-methylbut-2-enyl.

62. bis-Aromatic α,β-unsaturated ketones according claim 58 of the general formula XIX

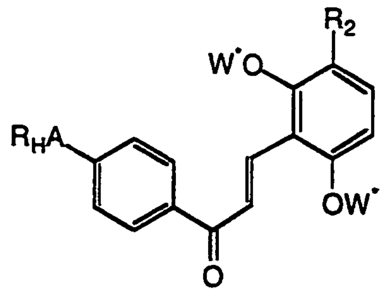


XIX

wherein R_2 and W^* are as defined in claim 48.

63. bis-Aromatic α,β-unsaturated ketones according to claim 61 or 62, in which R_2 designates propyl, prop-2-enyl, 1,1-dimethylpropyl, or 1,1-dimethylprop-2-enyl.

64. bis-Aromatic α,β-unsaturated ketones of the general formula XX

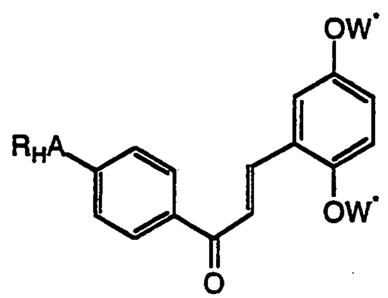


XX

wherein A, R_H and R_2 are as defined in claim 48.

65. bis-Aromatic α,β-unsaturated ketones according to claim 64, in which R_2 designates propyl, prop-2-enyl, 1,1-dimethylpropyl, 1,1-dimethylprop-2-enyl, 3-methylbutyl, or 3-methylbut-2-enyl.

66. bis-Aromatic α,β-unsaturated ketones of the general formula XXI



XXI

wherein A and R_H are as defined in claim 30, and W* is as defined in claim 48.

67. A method for the treatment or prophylaxis of a disease caused by a microorganism or a parasite in an animal, including a vertebrate, such as a bird, a fish or a mammal, including a human,

the microorganism or parasite being selected from

parasitic protozoa, in particular tissue and blood protozoa such as *Leishmania, Trypanosoma, Toxoplasma, Plasmodium, Pneumocystis, Babesia* and *Theileria;* intestinal protozoan flagellates such as *Trichomonas* and *Giardia;* intestinal protozoan *Coccidia* such as *Eimeria, Isospora, Cryptosporidium; Cappilaria, Microsporidium, Sarcocystis, Trichodina, Trichodinella, Dacthylogurus, Pseudodactylogurus, Acantocephalus, Ichthy-*

ophtherius, Botrecephalus; and intracellular bacteria, in particular *Mycobacterium, Legionella* species, *Listeria,* and *Salmonella,*

which method comprises administering to an animal an effective amount of an aromatic compound, or a prodrug thereof, which aromatic compound contains an alkylating site and which aromatic compound is capable of alkylating the thiol group in N-acetyl-L-cysteine at physiological pH.

68. A method according to claim 68, wherein the aromatic compound or, where appropriate, the prodrug thereof, shows any of the features as defined in any of claims 1-31.

69. A method according to claim 67 or 68, wherein an effective amount of a compound as defined in any of claims 32-66 is administered.

70. A method according to any of claims 67-69, in which the aromatic compound or the prodrug is administered in an amount of about 0.1-80 mg per kg body weight per day, preferably in an amount of about 0.5-20 mg per kg body weight per day.

71. A method according to any of claims 69-70, in which the aromatic compound or the prodrug is administered rectally in the form of suppositories.

72. A method according to any of claims 69-70, in which the aromatic compoundor prodrug is added to animal feed and/or drinking water such as poultry feed and/or drinking water.

73. A method according to any of claims 67-71, wherein the aromatic compound or the prodrug is administered in combination with another antiparasitic, antimycotic, antibiotic or antibabesial drug or anticoccidial agent or another drug against fish parasites.

74. A method according to claim 73, wherein the other antiparasitic drug is an antileishmanial drug selected from a pentavalent antimony-sodium gluconate, and allopurinol, or an anitmalarial drug selected from chloroquine and derivatives thereof, quinine, proguanil, cycloguanil, mefloquine, pyrimethamine, and artemisinin.

75. A method according to claim 73, wherein the other antibabesial drug is selected from quinuronium sulfate, pentamidine isethionate, imidocarb or diminazene, or the other anticoccidial drug is selected from sulfonamides, amprocid and coccidiostatic agents selected from ionomycins, such as monensin and salinomycin, or the other drug used against fish parasites is selected from benzimidazol and formaldehyde, or the additional antibiotic drug is an antituberculous drug selected from isoniazide, ethambutol, pyrazinamid, and rifampicin, or the additional antimycotic drug is selected from amphothericin B, muconar-

cidol, griseofluvin, and miconazol.

76. A pharmaceutical composition which contains an aromatic compound, or a prodrug thereof, which aromatic compound contains an alkylating site and which aromatic compound is capable of alkylating the thiol group in N-acetyl-L-cysteine at physiological pH, such as a compound as defined in any of claims 32-66 together with another antiparasitic, antimycotic, antibiotic or antibabesial drug or anticoccidial agent or another drug against fish parasites.

77. A pharmaceutical composition according to claim 76, wherein the other antiparasitic drug is an antileishmanial drug selected from a pentavalent antimony-sodium gluconate or allopurinol, or an antimalarial drug selected from chloroquine and derivatives thereof, quinine, proguanil, cycloguanil, mefloquine, pyrimethamine, and artemisinin.

78. A pharmaceutical composition according to claim 76, wherein the other antibabesial drug is selected from quinuronium sulfate, pentamidine isethionate, imidocarb or diminazene, or the other anticoccidial drug is selected from sulfonamides, amprocid and coccidiostatic agents selected from ionomycins, such as monensin and salinomycin, or the other drug used against fish parasites is selected from benzimidazol and formaldehyde, or the additional antibiotic drug is an antituberculous drug selected from isoniazide, ethambutol, pyrazinamid, and rifampicin, or the additional antimycotic drug is selected from amphothericin B, muconarcidol, griseofluvin, and miconazol.

79. A composition which contains an aromatic compound (or a prodrug) as defined in any of claims 32-66 in combination with an animal feed or drinking water for animals, or in combination with at least one pharmaceutical carrier or excipient suitable for administration to an animal, for the treatment or prophylaxis of a disease caused by a microorganism or a parasite selected from

parasitic protozoa, in particular tissue and blood protozoa such as *Leishmania, Trypanosoma, Toxoplasma, Plasmodium, Pneumocystis, Babesia* and *Theileria;* intestinal protozoan flagellates such as *Trichomonas* and *Giardia;* intestinal protozoan *Coccidia* such as *Eimeria, Isospora, Cryptosporidium; Cappilaria, Microsporidium, Sarcocystis, Trichodina, Trichodinella, Dacthylogurus, Pseudodactylogurus, Acantocephalus, Ichthyophtherius, Botrecephalus;* and intracellular bacteria, in particular *Mycobacterium, Legionella* species, *Listeria,* and *Salmonella.*

80. A composition according to claim 79 which is selected from a tablet, a suppository, and injection fluid.

81. A composition comprising a compound as defined in any of claims 48-66 in com-

bination with an animal feed or drinking water for animals, or in combination with at least one pharmaceutical carrier or excipient.

82. A composition according to claim 81 which is selected from a tablet, a suppository, and injection fluid.

83. A method for controlling transmission of parasitic diseases caused by parasites which have part of their life cycle in a vector, said method comprising applying an aromatic compounds, or a prodrug thereof, which aromatic compound contains an alkylating site and is capable of alkylating the thiol group in N-acetyl-L-cysteine at physiological pH, preferably a compound as defined in any of claims 32-66, to a locus which is a habitat of the vector so as to eradicate the parasites.

84. The method according to claim 83, wherein the application is performed by spraying a sprayable composition containing the aromatic compound.

85. A compound of the general formula I as defined in any of claims 32-36 with the proviso that W is a group -C≡C-.

86. A method for the preparation of a compound of the general formula I as defined in claim 32, said method comprising

a) for the preparation of a compound of the general formula I, in which both R are H, reacting a ketone of the general formula I′

$$X\text{-}Ar^1\text{-}CO\text{-}CH_3 \qquad I'$$

wherein X and Ar^1 are as defined in claim 32, with an aldehyde of the general formula I″

$$HCO\text{-}Ar^2\text{-}Y \qquad I''$$

wherein Ar^2 and Y are as defined in claim 32, or

b) for the preparation of a compound of the general formula I in which W is -C≡C-, reacting an activated derivative of a carboxylic acid of the general formula II′

$$X\text{-}Ar^1\text{-}COOH \qquad II'$$

wherein X and Ar^1 are as defined in claim 32,

with an ethyne derivative of the general formula II''

$$H\text{-}C{\equiv}C\text{-}Ar^2\text{-}Y \qquad II''$$

wherein Ar^2 and Y are as defined in claim 32, or

c) for the preparation of a compound of the general formula I, in which W is -CR=CR-, wherein R is as defined in claim 32, dehydrating a β-hydroxyketone of the general formula E,

$$X\text{-}Ar^1\text{-}CO\text{-}CRH\text{-}C(OH)R\text{-}Ar^2\text{-}Y \qquad E$$

wherein X, Y, Ar^1, Ar^2 and R are as defined in claim 32, or

d) for the preparation of a compound of the general formula I, wherein W is -C≡C-, eliminating HLea from a ketone of the general formula E1,

$$X\text{-}Ar^1\text{-}CO\text{-}CH{=}C(Lea)\text{-}Ar^2\text{-}Y \qquad E1$$

wherein X, Y, Ar^1 and Ar^2 are as defined in claim 32, and Lea is a halide or another leaving group such as hydroxy, alkoxy, tosyloxy, or trifluoromethanesulfonoxy, or

e) for the preparation of a compound of the general formula I, wherein W is -CR=CH-, wherein R is as defined in claim 32, reacting an aldehyde or ketone of the general formula F

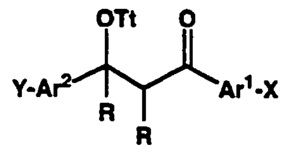
$$Y\text{-}Ar^2\text{-}CRO \qquad F$$

in which Y and Ar^2 are as defined in claim 32, with a phosphorus ylide (also called a phosphorane) of the general formula G,

$$T_3\text{-}P{=}CR\text{-}CO\text{-}Ar^1\text{-}X \qquad G$$

in which T is an aliphatic, alicyclic or aromatic group, and Ar^1, X and R are as defined in claim 32, or

f) for the preparation of a compound of the general formula I, in which W is -CR=CR- in which R is as defined in claim 32, eliminating HOTt from a ketone of the general formula K



K

wherein X, Y, Ar^1, Ar^2 and R are as defined in claim 32, and Tt is hydrogen, alkyl, tosyl, trifluoromethanesulfonyl or acyl, or

g) for the preparation of a compound of the general formula I, in which W is -CR=CR- in which R is as defined in claim 32, reacting a cinnamic acid of the general formula L

$$Y\text{-}Ar^2\text{-}CR{=}CR\text{-}COQ \qquad L$$

wherein Y, Ar^2 and R are as defined in claim 32, and Q is a hydroxy group, a carboxylate or a halogen atom, with an aromate of the general formula M

$$X\text{-}Ar^1 \qquad M$$

wherein X and Ar^1 are as defined in claim 32.

87. A method according to claim 85 c), in which the β-hydroxyketone of the general formula E is prepared by reacting an aldehyde of the general formula A

$$X\text{-}Ar^1\text{-}CHO \qquad A$$

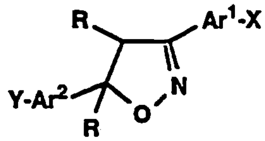
in which Ar^1 and X are as defined in claim 32, with hydroxylamine or a salt thereof to form the corresponding oxime of the general formula B

$$X\text{-}Ar^1\text{-}CH{=}N\text{-}OH \qquad B$$

reacting the oxime of the general formula B with a halogenating agent, eliminating hydrogen halide, and subsequently adding an olefin of the general formula C

$$CHR{=}CR\text{-}Ar^2\text{-}Y \qquad C$$

in which Ar^2, Y and R are as defined in claim 32, to form the corresponding isoxazoline of the formula D



D

reducing the isoxazoline of the formula D and hydrolyzing the reduction product to form a β-hydroxyketone of the general formula E.

88. A method according to claim 86 d), in which the β-hydroxyketone of the general formula E1 is prepared by reacting an aldehyde of the general formula A

X-Ar^1-CHO A

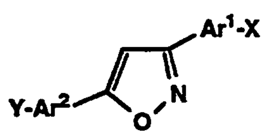
in which Ar^1 and X are as defined in claim 32, with hydroxylamine or a salt thereof to form the corresponding oxime of the general formula B

X-Ar^1-CH=N-OH B

reacting the oxime of the general formula B with a halogenating agent and subsequently adding an acetylene of the general formula C1

CH≡C-Ar^2-Y C1

in which Ar^2, Y and R are as defined in claim 32, to form the corresponding isoxazole of the formula D1



D1

reducing the isoxazole of the formula D1 and hydrolysing the reduction product.

89. A method according to claim 86 c) or d) wherein the β-hydroxyketone of the general formula E or E1 is prepared *in situ* by reduction and hydrolysis of the isoxazoline D or isoxazole D1 in one step.

90. A method according to claim 86 f) wherein the ketone of the general formula K in

which Tt is hydrogen, is prepared by reacting a compound of the general formula H

$$X\text{-}Ar^1\text{-}CV{=}CH_2 \qquad H$$

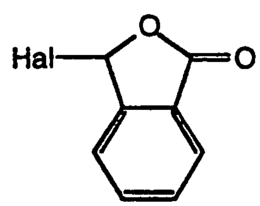
in which X and Ar^1 are as defined in claim 32, and in which V represents a secondary amine group, such as a morpholino or piperidino group, with an aldehyde of the general formula J

$$Y\text{-}Ar^2\text{-}CHO \qquad J$$

in which Y and Ar^2 are as defined in claim 32, to form an intermediate which is hydrolyzed to reconstitute the carbonyl function.

92. A method for preparing a compound of the general formula I as defined in claim 32, wherein Z is a group D or E as defined in claim 33, comprising reacting the corresponding compound of the general formula I wherein X and/or Y is AZ wherein A is as defined in claim 32, in particular -O- or -NH-, and Z is H, with the appropriate halide of the general fomula D-Hal or E-Hal

$$Hal\text{-}CR^{*}R^{**}\text{-}O\text{-}CO\text{-}R''' \qquad D\text{-}Hal$$



E-Hal

in which R^{*}, R^{**} and R''' are as defined in claim 33, and Hal is a halogen atom such as chlorine, bromine or iodine.

93. A method according to claim 92, wherein the halide D-Hal is iodomethyl pivaloylate.

94. A method for preparing a compound of the general formula I as defined in claim 32, wherein Z is a carboxylic acid residue (A) as defined in claim 33, comprising reacting the corresponding compound of the general formula I wherein X and/or Y is AZ, wherein A is as defined in claim 32, and Z is H, with a reactive derivative of the carboxylic acid HO-CO-R', wherein R' is as defined in claim 33, the reactive acid derivate being, in particular, selected from activated esters, anhydrides, and acid halides, such as the acid chloride.

95. A method for preparing a compound of the general formula I as defined in claim 32, wherein Z is a dimethylcarbamoyl group, comprising reacting the corresponding com-

pound of the general formula I, in which X and/or Y is AZ, wherein A is as defined in claim 32, and Z is H, with an activated derivative of N,N-dimethylcarbamic acid.

96. A method for preparing a compound of the general formula I as defined in claim 32, wherein Z is an alkoxyalkyl group (C) as defined in claim 33, comprising reacting the corresponding compound of the general formula I, in which X and/or Y is AZ, wherein A is as defined in claim 32, and Z is H, with an alkyl-α-haloalkyl ether.

97. A method for preparing a compound of the general formula I as defined in claim 32, wherein R is halogen comprising, reacting the corresponding compound of the general formula I in which W is -CH=CH- with bromine followed by dehydrobromination of the formed dibromide using potassium acetate in methanol.

AMENDED CLAIMS

[received by the International Bureau on 05 August 1993 (05.08.93); original claims 48,49,51,53,54 and 57 amended; new claims 67-73 added; claims 67-96 renumbered as claims 74-103; other claims unchanged (27 pages)]

1. The use of an aromatic compound, or a prodrug thereof, which aromatic compound contains an alkylating site and which aromatic compound is capable of alkylating the thiol group in N-acetyl-L-cysteine at physiological pH, for the preparation of a pharmaceutical composition or a medicated feed, food or drinking water for the treatment or prophylaxis of a disease caused by a microorganism or a parasite in an animal, including a vertebrate, such as a bird, a fish or a mammal, including a human,

the microorganism or parasite being selected from

parasitic protozoa, in particular tissue and blood protozoa such as *Leishmania, Trypanosoma, Toxoplasma, Plasmodium, Pneumocystis, Babesia* and *Theileria;* intestinal protozoan flagellates such as *Trichomonas* and *Giardia;* intestinal protozoan *Coccidia* such as *Eimeria, Isospora, Cryptosporidium; Cappilaria, Microsporidium, Sarcocystis, Trichodina, Trichodinella, Dacthylogurus, Pseudodactylogurus, Acantocephalus, Ichthyophtherius, Botrecephalus;* and intracellular bacteria, in particular *Mycobacterium, Legionella* species, *Listeria,* and *Salmonella.*

2. The use according to claim 1, wherein the aromatic compound, in a concentration in which it causes less than 50% reduction of the thymidine uptake by human lymphocytes in the Lymphocyte Proliferation Assay using phytohemagglutinin (PHA), meets at least one of the following criteria:

a) the aromatic compound is capable of inhibiting *in vitro* the growth or multiplication of *Leishmania major* promastigotes by at least 80%, as determined by uptake of tritiated thymidine,

b) the aromatic compound is capable of inhibiting *in vitro* the growth or multiplication of *Plasmodium falciparum* by at least 80%, as determined by uptake of tritiated hypoxanthine,

c) the aromatic compound is capable of inhibiting *in vitro* the growth or multiplication of *Eimeria tenella* in chicken fibroblast cell cultures by at least 70%, as determined by counting the parasites,

d) the aromatic compound is capable of inhibiting *in vitro* the growth or multiplication of *Mycobacterium tuberculosis* or *Legionella pneumophila* by at least 50%, as determined by colony counts.

3. The use according to claim 2, wherein the aromatic compound meets all of the criteria

a) to d).

4. The use according to claim 2, wherein the aromatic compound, in a concentration in which it causes less than 40% reduction of the thymidine uptake by human lymphocytes in the Lymphocyte Proliferation Assay using PHA, meets at least one of the criteria a) to d).

5. The use according to claim 4, wherein the aromatic compound meets all of the criteria a) to d).

6. The use according to claim 2, wherein the aromatic compound, in a concentration in which it causes less than 20% reduction of the thymidine uptake by human lymphocytes in the Lymphocyte Proliferation Assay using PHA, meets at least one of the criteria a) to d).

7. The use according to claim 6, wherein the aromatic compound meets all of the criteria a) to d).

8. The use according to claim 1, wherein the pharmaceutical composition is a composition for the treatment or prophylaxis of diseases caused by *Leishmania* in humans or dogs, and the aromatic compound is capable of inhibiting *in vitro* the growth of *Leishmania major* promastigotes by at least 80%, as determined by uptake of tritiated thymidine, in a concentration of the compound in which it causes less than 50% reduction of the thymidine uptake by human lymphocytes in the Lymphocyte Proliferation Assay using PHA.

9. The use according to claim 8, wherein the aromatic compound is capable of inhibiting *in vitro* the growth of *Leishmania major* promastigotes by at least 80%, as determined by uptake of tritiated thymidine, in a concentration of the compound in which it causes less than 40% reduction, preferably less than 20% reduction, of the thymidine uptake by human lymphocytes in the Lymphocyte Proliferation Assay using PHA.

10. The use according to claim 1, wherein the pharmaceutical composition is a composition for the treatment or prophylaxis of diseases caused by *Leishmania* in humans or dogs, and the aromatic compound, or the prodrug, when administered intraperitoneally in the *in vivo* test described in Example 8 herein in a dose of up to 20 mg per kg body weight once daily for 40 days to female BALB/c mice which have been infected with *L. major* (10^7/mouse), the administration being initiated one week after infection, is capable of preventing increase in lesion size by at least 60%, preferably at least 80%, more preferably at least 90%.

11. The use according to claim 10, wherein the aromatic compound, or the prodrug, when

administered intraperitoneally in the *in vivo* test described in Example 8 herein in a dose of up to 10 mg per kg body weight once daily for 40 days to female BALB/c mice which have been infected with *L. major* (10^7/mouse), the administration being initiated one week after infection, is capable of preventing increase in lesion size by at least 60%, preferably at least 80%, more preferably at least 90%.

12. The use according to claim 1, wherein the pharmaceutical composition is a composition for the treatment or prophylaxis of diseases caused by *Leishmania* in humans or dogs, and the aromatic compound, or the prodrug, when administered intraperitoneally in the *in vivo* test described in Example 9 herein in a dose of up to 20 mg per kg body weight two times daily for 7 days to male Syrian golden hamsters which have been infected with *L. donovani* promastigotes (2 x 10^7/hamster), the administration being initiated one day after infection, is capable of reducing the parasite load in the liver of the hamsters by at least 60%, preferably by at least 80%, and more preferably by at least 90%.

13. The use according to claim 12, wherein the aromatic compound when administered intraperitoneally in the *in vivo* test described in Example 9 herein in a dose of up to 10 mg per kg body weight two times daily for 7 days to male Syrian golden hamsters which have been infected with *L. donovani* promastigotes (2 x 10^7/hamster), the administration being initiated one day after infection, is capable of reducing the parasite load in the liver of the hamsters by at least 60%, preferably by at least 80%, and more preferably by at least 90%.

14. The use according to claim 1, wherein the pharmaceutical composition is a composition for the treatment or prophylaxis of malaria caused by *Plasmodium spp.* in humans, and the aromatic compound is capable of inhibiting *in vitro* the growth of *Plasmodium falciparum* by at least 80%, as measured by uptake of tritiated hypoxanthine, in a concentration of the compound in which it causes less than 50% reduction of the thymidine uptake by human lymphocytes, as measured by the Lymphocyte Proliferation Assay using PHA.

15. The use according to claim 14, wherein the aromatic compound is capable of inhibiting *in vitro* the growth of *Plasmodium falciparum* by at least 80% in a concentration of the compound in which it causes less than 40% reduction, preferably less than 20% reduction, of the thymidine uptake by human lymphocytes, as measured by the Lymphocyte Proliferation Assay using PHA.

16. The use according to claim 1, wherein the pharmaceutical composition is a composition for the treatment or prophylaxis of diseases caused by *Plasmodium spp.* in humans, and the aromatic compound, when administered intraperitoneally in the *in vivo* test described in Example 16 herein in a dose of up to 20 mg per kg body weight two

times daily for 6 days to female BALB/c mice which have been infected with malaria *P. yoelii* (2×10^5/mouse), the administration being initiated one day after infection, is able to prevent increase in the parasitemia during the administration period.

17. The use according to claim 16, wherein the aromatic compound, or the prodrug, when administered intraperitoneally in the *in vivo* test described in Example 16 herein in a dose of up to 20 mg per kg body weight two times daily for 10 days to 8 weeks old female BALB/c mice which have been infected with malaria *P. yoelii* (2×10^5/mouse), the administration being initiated one day after infection, is capable of clearing the parasite from the mice within at the most 23 days.

18. The use according to claim 17, wherein the aromatic compound, or the prodrug, when administered intraperitoneally in the *in vivo* test described in Example 16 herein in a dose of up to 20 mg per kg body weight two times daily for 8 days to 8 weeks old female BALB/c mice which have been infected with malaria *P. yoelii* strain YM (1×10^6/mouse), the administration being initiated one day after infection, is capable of clearing the parasite from the mice within at the most 21 days, preferably within at the most 17 days.

19. The use according to claim 18, wherein the aromatic compound, or the prodrug, when administered intraperitoneally in the *in vivo* test described in Example 16 herein in a dose of 5 mg per kg body weight two times daily for 10 days to 8 weeks old female BALB/c mice which have been infected with malaria *P. yoelii* (2×10^5/mouse), the administration being initiated one day after infection, is capable of clearing the parasite from the mice within at the most 23 days.

20. The use according to claim 19, wherein the aromatic compound, or the prodrug, when administered intraperitoneally in the *in vivo* test described in Example 16 herein in a dose of 5 mg per kg body weight two times daily for 8 days to 8 weeks old female BALB/c mice which have been infected with malaria *P. yoelii* strain YM (1×10^6/mouse), the administration being initiated one day after infection, is capable of clearing the parasite from the mice within at the most 21 days, preferably within at the most 17 days.

21. The use according to claim 1 of an aromatic compound, or a prodrug thereof, which aromatic compound contains an alkylating site and which aromatic compound is capable of alkylating the thiol group in N-acetyl-L-cysteine at physiological pH, for the preparation of a pharmaceutical composition or a medicated feed or drinking water for the treatment or propylaxis of diseases caused by *Coccidia* in poultry such as chickens or turkeys, wherein the aromatic compound, or the prodrug, when administered to chickens with the feed in a concentration of up to 400 ppm for at most 28 days in the *in vivo* test described in Example 28 herein, is capable of controlling infection by *Eimeria tenella* in at least 60% of the chickens and preventing pathological alterations in at least 50% of the

chickens.

22. The use according to claim 21, wherein the aromatic compound, when administered to chickens with the feed in a concentration of up to 120 ppm for at most 28 days in the *in vivo* test described in Example 28 herein, is capable of controlling infection by *Eimeria tenella* in at least 60% of the chickens and preventing pathological alterations in at least 65% of the chickens.

23. The use according to claim 1, wherein the pharmaceutical composition is a composition for the treatment or prophylaxis of diseases caused by intracellular bacteria such as *Mycobacteria* in humans or animals such as cattle, and the aromatic compound is one which is capable of inhibiting the growth and multiplication of *Mycobacteria tuberculosis in vitro* in the test described in Example 17 herein at a mean MIC of 10 μg per ml, and, in the same concentration, causes less than 50% reduction of the thymidine uptake of human lymphocytes as measured by The Lympocyte Proliferation Assay.

24. The use according to claim 1, wherein the pharmaceutical composition is a composition for the treatment or prophylaxis of diseases caused by intracellular bacteria such as *Legionella* in humans, and the aromatic compound is one which is capable of inhibiting the growth and multiplication of *Legionella pneumophila in vitro* in the test described in Example 17 herein at a mean MIC of 10 μg per ml, and, in the same concentration, causes less than 50% reduction of the thymidine uptake of human lymphocytes as measured by The Lympocyte Proliferation Assay.

25. The use according to any of the preceding claims, in which the aromatic compound contains an aromatic ring attached to the alkylating site.

26. The use according to any of claims 1-25, in which the compound has electron-donating groups attached to an aromatic ring.

27. The use according to claim 25, wherein the alkylating site is a double or triple bond conjugated with a carbonyl group which carbonyl group optionally is further conjugated with an aromatic ring such as a phenyl group.

28. The use according to any of claims 25, wherein the aromatic ring attached to the alkylating site contains at least one electron donating group such as an oxygen, nitrogen or sulphur function.

29. The use according to claim 26, wherein the electron donating group(s) is/are attached to the aromatic ring in a position next to and/or most remote relative to the position through which the aromatic ring is attached to the alkylating site.

30. The use according to any of claims 1-7, wherein the disease is human leishmaniasis caused by *Leishmania donovani*, *L. infantum*, *L. aethiopica*, *L. major*, *L. tropica*, *L. mexicana complex*, or *L. braziliensis complex* or human malaria caused by *Plasmodium falciparum*, *P. ovale*, *P. vivax*, or *P. malariae*.

31. The use according to any of claims 1-7, wherein the disease is a parasitic disease in livestock, such as *Babesia* in cattle, or a parasitic disease in birds, such as a disease caused by *Coccidia* such as *Eimeria tenella* in poultry such as chicken or turkey, or a parasitic disease in fish, such as *Pseudodactylogurus* or *Trichodina*.

32. The use according to any of the preceding claims, wherein the aromatic compound is a bis-aromatic α,β-unsaturated ketone of the general formula I

$$X_m\text{-}Ar^1\text{-}CO\text{-}W\text{-}Ar^2\text{-}Y_n \qquad \text{I}$$

wherein

W is either -CR=CR- or -C≡C-, wherein each R independently of the other R designates hydrogen, C_{1-3} alkyl, or halogen,

Ar^1 and Ar^2 are the same or different and each designate an aromate selected from phenyl and 5- or 6-membered unsaturated heterocyclic rings containing one, two or three hetero-atoms selected from oxygen, sulfur, and nitrogen, such as furanyl, thiophenyl, pyrrolyl, imidazolyl, isoxazolyl, oxazolyl, thiazolyl, pyrazolyl, pyridinyl, or pyrimidinyl, which aromate may be substituted with one or more substituents selected from

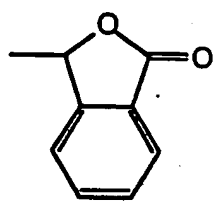
> halogen; nitro; nitroso; and C_{1-12}, preferably C_{1-6}, straight or branched aliphatic hydrocarbyl which may be saturated or may contain one or more unsaturated bonds selected from double bonds and triple bonds, which hydrocarbyl may be substituted with one or more substituents selected from hydroxy, halogen, amino, and amino which is optionally alkylated with one or two C_{1-6} alkyl groups;

Y and X are the same or different and each designate a group AR_H or a group AZ, wherein A is -O-, -S-, -NH-, or -N(C_{1-6} alkyl)-, R_H designates C_{1-6} straight or branched aliphatic hydrocarbyl which may be saturated or may contain one or more unsaturated bonds selected from double bonds and triple bonds, and Z designates H or (when the compound is a prodrug) a masking group which is readily decomposed under conditions prevailing in the animal body to liberate a group AH, in which A is as defined abov; m designates 0, 1 or 2, and n designates 0, 1, 2 or 3, whereby, when m is 2, then the two groups X are the same or different, and when n is 2 or 3, then the two or three groups Y

are the same or different, with the proviso that n and m are not both 0.

33. The use according to claim 32, wherein Z, when designating a masking group, is selected from the below groups (A)-(E)

-CO-R′	(A)
$-CON(CH_3)_2$	(B)
-CR*R**-O-R″	(C)
-CR*R**-O-CO-R‴	(D)



(E)

wherein R* and R** each independently designate hydrogen or C_{1-3} alkyl, R′, R″ and R‴ each designate C_{1-6} alkyl or is an aromate Ar^1 or Ar^2 as defined in claim 32.

34. The use according to claims 32 and 33, wherein Ar^1, or Ar^2 or both independently are phenyl or an aromatic 5- or 6-membered heterocyclic ring containing one, two or three heteroatoms selected from oxygen, sulphur and nitrogen, n is 0, 1, 2, or 3, m is 0, 1 or 2, at least one of the groups X is in a position in Ar^1 most remote relative to and/or next to the position through which Ar^1 is bound to the carbonyl group, and at least one of the groups Y is in a position in Ar^2 most remote relative to and/or next to the position through which Ar^2 is bound to W.

35. The use according to claim 32, in which A is O.

36. The use according to claim 33, in which Z is pivaloyl, pivaloyloxymethyl or N,N-dimethylcarbamoyl.

37. The use according to any of claims 32-36, in which the compound of formula I is a compound of formula II

$$X_m\text{-Ph-C(O)-CH=CH-Ph-}Y_n \qquad \text{II}$$

wherein Ph designates phenyl, and X_m and Y_n are as defined in claim 32, and each phenyl group may be substituted with one or more substituents selected from halogen; nitro; nitroso; and C_{1-12}, preferably C_{1-6}, straight or branched aliphatic hydrocarbyl which may

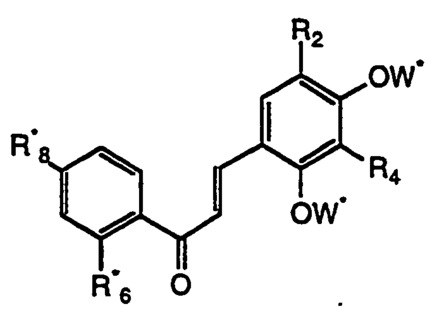
be saturated or may contain one or more unsaturated bonds selected from double bonds and triple bonds, which hydrocarbyl may be substituted with one or more substituents selected from hydroxy, halogen, amino, and amino which is optionally alkylated with one or two C_{1-6} alkyl groups.

38. The use according to claim 37, in which X and/or Y is OH or a group OR_H, in which R_H is as defined in claim 32, or OZ^*, in which Z^* is a masking group which is readily decomposed under conditions prevailing in the animal body to liberate the group OH, in particular one of the groups (A)-(E) as defined in claim 33, preferably pivaloyl, pivaloyloxymethyl or N,N-dimethylcarbonyl.

39. The use according to claim 37, wherein the substituent or substituents on the phenyl groups is/are C_{1-12}, preferably C_{1-6}, straight or branched aliphatic hydrocarbyl which may be saturated or may contain one or more unsaturated bonds selected from double bonds and triple bonds, which hydrocarbyl may be substituted with one or more substituents selected from hydroxy, halogen, amino, and amino which is optionally alkylated with one or two C_{1-6} alkyl groups.

40. The use according to claim 39, wherein the substituent or substituents on the phenyl groups is/are methyl, ethyl, propyl, isopropyl, tert.-butyl, prop-2-enyl, 1,1-dimethylpropyl, 1,1-dimethylprop-2-enyl, 3-methylbutyl, or 3-methylbut-2-enyl.

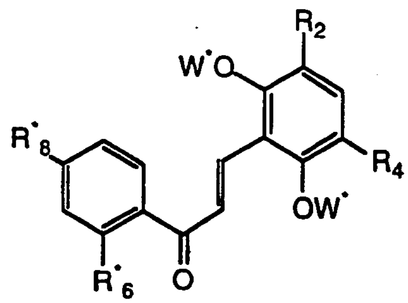
41. The use according to claim 37, wherein the bis-aromatic α,β-unsaturated ketone has the general formula III



III

wherein R_2 and R_4 designate R_H as defined in claim 32 or H, one of R^*_6 and R^*_8 designate OW^* and the other is H, or both R^*_6 and R^*_8 designate H, and W^* designates H, R_H or a group (A)-(E) as defined in claim 33 wherein both R^* and R^{**} designate H.

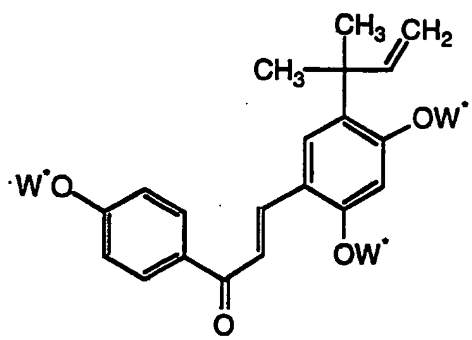
42. The use according to claim 37, wherein the bis-aromatic α,β-unsaturated ketone has the general formula IV



IV

wherein R_2, R_4, R^*_6, R^*_8 and OW* are as defined in claim 41.

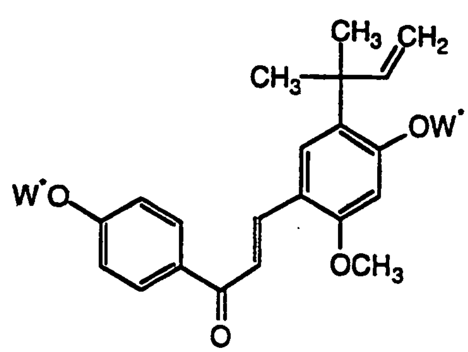
43. The use according to claim 37, in which the bis-aromatic α,β-unsaturated ketone has the general formula V



V

wherein W* is as defined in claim 41.

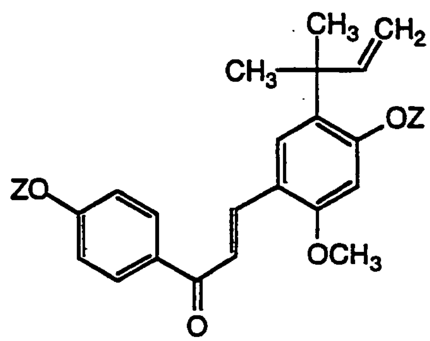
44. The use according to claim 37, in which the bis-aromatic α,β-unstaturated ketone has the general formula VI



VI

wherein W* is as defined in claim 41.

45. The use according to claim 37 in which the bis-aromatic α,β-unsaturated ketone has the general formula VII

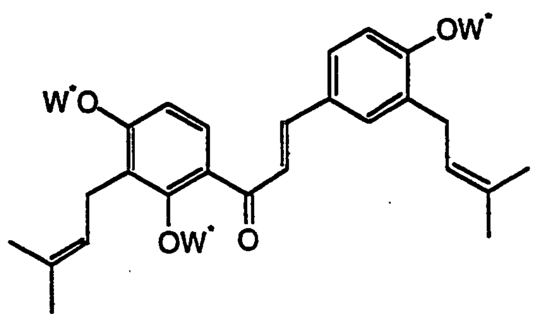


VII

wherein Z is as defined in claim 33.

46. The use according to claim 45, wherein Z designates pivaloyl, pivaloyloxymethyl or N,N-dimethylcarbonyl.

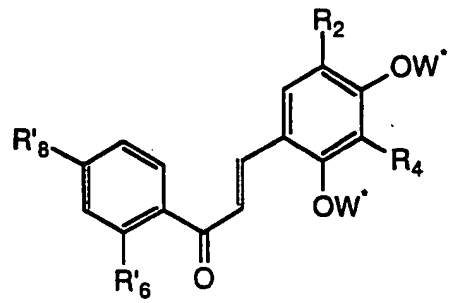
47. The use according to claim 37 in which the bis-aromatic α,β-unsaturated ketone has the general formula VIII



VIII

wherein W* is as defined in claim 41.

48. bis-Aromatic α,β-unsaturated ketones of the general formula IX

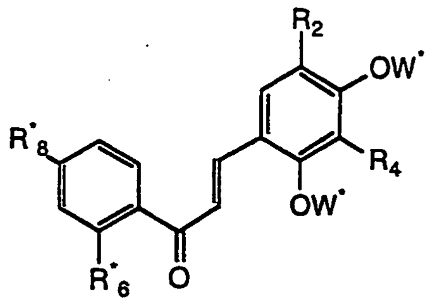


IX

wherein R_2 and R_4 designates R_H or H, where R_H designates C_{1-6} straight or branched aliphatic hydrocarbyl which may be saturated or may contain one or more unsaturated bonds selected from double bonds and triple bonds, R'_6 designates $A(W^*)_p$ and R'_8 designates H, wherein W^* designates R_H or a masking group Z as defined in claim 32 or 33, and A designates S, N or O, whereby, when A designates S or O, then p designates 1, and when A designates N, then p designates 2, or R'_8 designates $A(W^*)_p$ and R'_6 designates H, wherein W^* designates H, R_H or a masking group Z as defined in claim 32 or 33, A designates S, N or O, whereby, when A designates S or O, then p designates 1, and when A designates N, then p designates 2, or both R'_6 and R'_8 designate H, with the proviso that when R_2 and R_4 both are H, then at least one W^* designates a masking group Z as defined in claim 32 or 33, whereby when the masking group is a group -CO-R', then R' is C_{2-6} alkyl or is an aromate Ar^1 or Ar^2 as defined in claim 32, and R_H is as defined in claim 32,

with the exception of licochalcone A, licochalcone C, 3-[4-hydroxy-5-(1,1-dimethylprop-2-enyl)-2-methoxyphenyl]-1-[4-(methoxymethoxy)phenyl]-2-propen-1-one, 3-[4-acetyloxy-5-(1,1-dimethylprop-2-enyl)-2-methoxyphenyl]-1-[4-(methoxymethoxy)phenyl]-2-propen-1-one, 3-[5-(1,1-dimethylprop-2-enyl)-2,4-dimethoxyphenyl]-1-[4-(methoxy)phenyl]-2-propen-1-one, 3-[4-acetyloxy-5-(1,1-dimethylprop-2-enyl)-2-methoxyphenyl]-1-(4-acetyloxyphenyl)-2-prop-1-one, 3-[2-hydroxy-4-methoxy-3-(3-methylbut-2-enyl)phenyl]-1-[4-[(3,7,11-trimethyl-2,6-dodecatri-10-enyl)oxy]phenyl]-2-prop-1-one, 2,4-dihydroxy-3-methylchalcone, and 1-[4-(methocymethoxy)phenyl]-3-[2-methoxy-4-[3-(methyl-2-butenyl)oxy]-2-propenone-1.

49. bis-Aromatic α,β-unsaturated ketones according to claim 48 having the general formula X

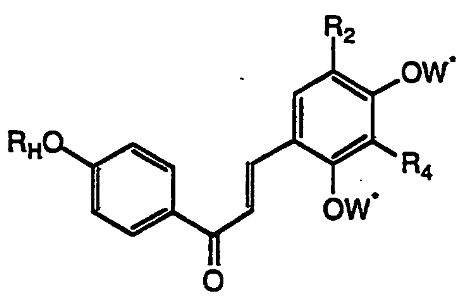


X

wherein R_2 and R_4 are as defined in claim 48, R^*_8 designates OW^* and R^*_6 designates H, wherein W^* designates H, R_H or a masking group Z as defined in claim 32 or 33, or R^*_6 designates OW^* and R^*_8 designates H, wherein W^* designates R_H or a masking group Z as defined in claim 32 or 33, or both designate H with the proviso that when both R_2 and R_4 are H, then at least one W^* designates a masking group Z as defined in claim 32 or 33, whereby when the masking group is a group -CO-R', then R' is C_{2-6} alkyl or is an aromate Ar^1 or Ar^2 as defined in claim 32.

50. bis-Aromatic α,β-unsaturated ketones according to claim 49, wherein R_4 designates H.

51. bis-Aromatic α,β-unsaturated ketones according to claim 48 of the general formula XI

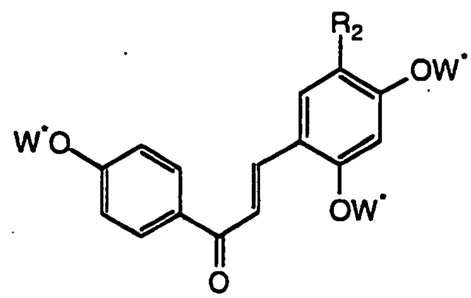


XI

wherein R_2, R_4 and W^* are as defined in claim 48 with the proviso that when both R_2 and R_4 are H, then at least one W^* designates a masking group Z as defined in claim 32 or 33, whereby when the masking group is a group -CO-R', then R' is C_{2-6} alkyl or is an aromate Ar^1 or Ar^2 as defined in claim 32.

52. bis-Aromatic α,β-unsaturated ketones according to claim 48, 49 or 50, in which R_2 and/or R_4 designates methyl, ethyl, propyl, isopropyl, tert.-butyl, prop-2-enyl, 1,1-dimethylpropyl, 1,1-dimethylprop-2-enyl, 3-methylbutyl, or 3-methylbut-2-enyl.

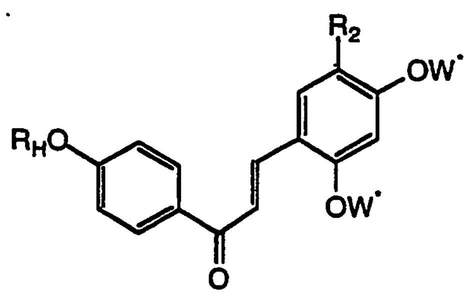
53. bis-Aromatic α,β-unsaturated ketones according to claim 48 of the general formula XII



XII

wherein R_2 and W* are as defined in claim 48 with the proviso that when R_2 is H, then at least one W* designates a masking group Z as defined in claim 32 or 33, whereby when the masking group is a group -CO-R′, then R′ is C_{2-6} alkyl or is an aromate Ar^1 or Ar^2 as defined in claim 32.

54. bis-Aromatic α,β-unsaturated ketones according to claim 53 of the general formula XIII

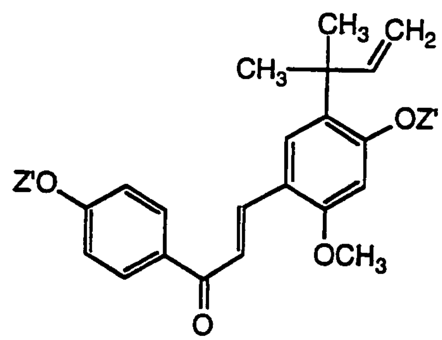


XIII

wherein R_H is as defined in claim 32, and R_2 and W* are as defined in claim 48 with the proviso that when R_2 is H, then at least one W* designates a masking group Z as defined in claim 32 or 33, whereby when the masking group is a group -CO-R′, then R′ is C_{2-6} alkyl or is an aromate Ar^1 or Ar^2 as defined in claim 32.

55. bis-Aromatic α,β-unsaturated ketones according to claim 53 or 54, in which R_2 designates methyl, ethyl, propyl, isopropyl, tert.-butyl, prop-2-enyl, 1,1-dimethylpropyl, 1,1-dimethylprop-2-enyl, 3-methylbutyl, or 3-methylbut-2-enyl.

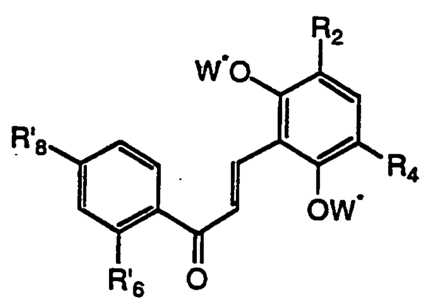
56. bis-Aromatic α,β-unsaturated ketones according to claim 48 of the general formula XIV



XIV

wherein Z′ is one of the groups (A)-(E) as defined in claim 33.

57. bis-Aromatic α,β-unsaturated ketones of the general formula XV

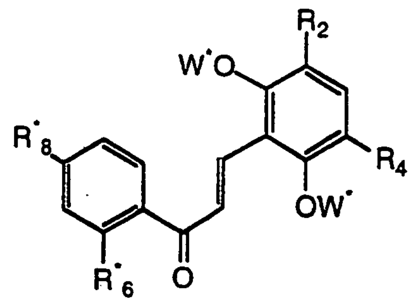


XV

wherein R'_6 designates $A(W^*)_p$ and R'_8 designates H, wherein W^* designates R_H or a masking group Z as defined in claim 32 or 33, A designates S, N or O, whereby, when A sesignates S or O, then p designates 1, and when A designates N, then p designates 2, or R'_8 designates $A(W^*)_p$ and R'_6 designates H, wherein W^* designates H, R_H or a masking group Z as defined in claim 32 or 33, A designates S, N or O, whereby, when A sesignates S or O, then p designates 1, and when A designates N, then p designates 2, or both R'_6 and R'_8 designate H, and R_H is as defined in claim 32,

with the exception of 2,6-methoxychalcone and 2-hydroxy-6-methoxychalcone.

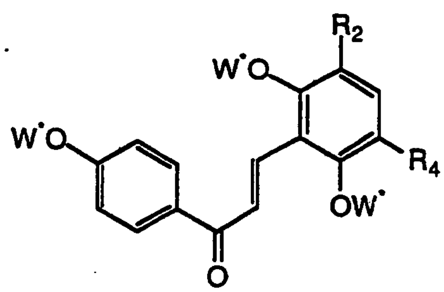
58. bis-Aromatic α,β-unsaturated ketones of the general formula XVI



XVI

wherein R_2, R_4 and W* are as defined in claim 48, and R^*_8 and R^*_6 are as defned in claim 49.

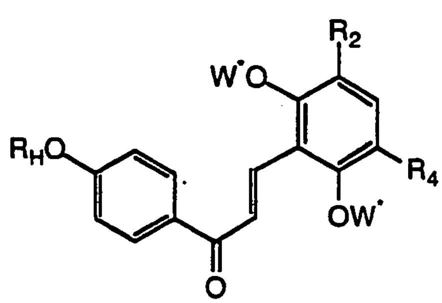
59. bis-Aromatic α,β-unsaturated ketones according to claim 58 of the general formula XVII



XVII

wherein R_2, R_4 and W* are as defined in claim 48.

60. bis-Aromatic α,β-unsaturated ketones according to claim 58 of the general formula XVIII

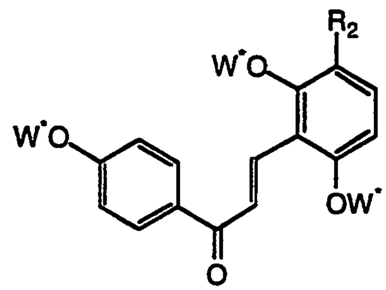


XVIII

wherein R_H is as defined in claim 32, R_2, R_4 and W* are as defined in claim 48.

61. bis-Aromatic α,β-unsaturated ketones according to any of claims 57-60, in which R_2 and/or R_4 designates methyl, ethyl, propyl, isopropyl, tert.-butyl, prop-2-enyl, 1,1-dimethylpropyl, 1,1-dimethylprop-2-enyl, 3-methylbutyl, or 3-methylbut-2-enyl.

62. bis-Aromatic α,β-unsaturated ketones according claim 58 of the general formula XIX

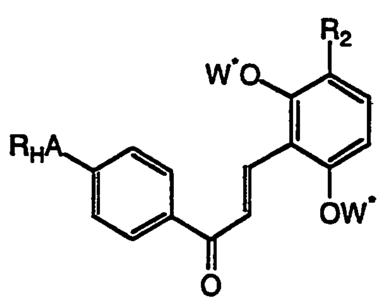


XIX

wherein R_2 and W* are as defined in claim 48.

63. bis-Aromatic α,β-unsaturated ketones according to claim 61 or 62, in which R_2 designates propyl, prop-2-enyl, 1,1-dimethylpropyl, or 1,1-dimethylprop-2-enyl.

64. bis-Aromatic α,β-unsaturated ketones of the general formula XX

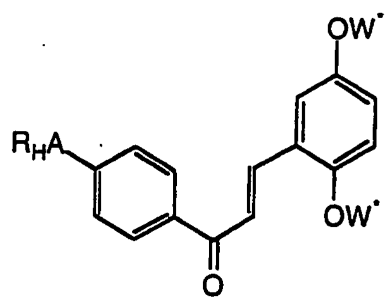


XX

wherein A, R_H and R_2 are as defined in claim 48.

65. bis-Aromatic α,β-unsaturated ketones according to claim 64, in which R_2 designates propyl, prop-2-enyl, 1,1-dimethylpropyl, 1,1-dimethylprop-2-enyl, 3-methylbutyl, or 3-methylbut-2-enyl.

66. bis-Aromatic α,β-unsaturated ketones of the general formula XXI



XXI

wherein R_H is as defined in claim 32, and W^* and A is as defined in claim 48.

67. 3,5-Dimethoxy-4'-prop-2-enyloxychalcone.

68. 2,3-Dimethoxy-4'-prop-2-enyloxychalcone.

69. 3,4-Dimethoxy-4'-prop-2-enyloxychalcone.

70. 2,4-Dimethoxy-4'-(prop-2-enyloxy)-chalcone,
2,4-diethoxy-4'-(prop-2-enyloxy)-chalcone,
2,4-di-n-propoxy-4'-(prop-2-enyloxy)-chalcone,
2,4-diisopropoxy-4'-(prop-2-enyloxy)-chalcone,
2,4-di-n-butoxy-4'-(prop-2-enyloxy)-chalcone,
2,4-di-t-butoxy-4'-(prop-2-enyloxy)-chalcone,
2,4-dimethoxy-5-methyl-4'-(prop-2-enyloxy)-chalcone,
2,4-diethoxy-5-methyl-4'-(prop-2-enyloxy)-chalcone,
2,4-di-n-propoxy-5-methyl-4'-(prop-2-enyloxy)-chalcone,
2,4-diisopropoxy-5-methyl-4'-(prop-2-enyloxy)-chalcone,
2,4-di-n-butoxy-5-methyl-4'-(prop-2-enyloxy)-chalcone,
2,4-di-t-butoxy-5-methyl-4'-(prop-2-enyloxy)-chalcone,
2,4-dimethoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)-chalcone,
2,4-diethoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)-chalcone,
2,4-di-n-propoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)-chalcone,
2,4-diisopropoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)-chalcone,
2,4-di-n-butoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)-chalcone,
2,4-di-t-butoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)-chalcone,
2,4-dimethoxy-5-propyl-4'-(prop-2-enyloxy)-chalcone,
2,4-diethoxy-5-propyl-4'-(prop-2-enyloxy)-chalcone,
2,4-di-n-propoxy-5-propyl-4'-(prop-2-enyloxy)-chalcone,

2,4-diisopropoxy-5-propyl-4'-(prop-2-enyloxy)-chalcone,
2,4-di-n-butoxy-5-propyl-4'-(prop-2-enyloxy)-chalcone,
2,4-di-t-butoxy-5-propyl-4'-(prop-2-enyloxy)-chalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)-chalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)-chalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)-chalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)-chalcone,
2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)-chalcone,
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)-chalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)-chalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)-chalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)-chalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)-chalcone,
2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)-chalcone, or
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)-chalcone.

71. 2,6-Dimethoxy-4'-(prop-2-enyloxy)-chalcone,
2,6-diethoxy-4'-(prop-2-enyloxy)-chalcone,
2,6-di-n-propoxy-4'-(prop-2-enyloxy)-chalcone,
2,6-diisopropoxy-4'-(prop-2-enyloxy)-chalcone,
2,6-di-n-butoxy-4'-(prop-2-enyloxy)-chalcone,
2,6-di-t-butoxy-4'-(prop-2-enyloxy)-chalcone,
2,6-dimethoxy-5-methyl-4'-(prop-2-enyloxy)-chalcone,
2,6-diethoxy-5-methyl-4'-(prop-2-enyloxy)-chalcone,
2,6-di-n-propoxy-5-methyl-4'-(prop-2-enyloxy)-chalcone,
2,6-diisopropoxy-5-methyl-4'-(prop-2-enyloxy)-chalcone,
2,6-di-n-butoxy-5-methyl-4'-(prop-2-enyloxy)-chalcone,
2,6-di-t-butoxy-5-methyl-4'-(prop-2-enyloxy)-chalcone,
2,6-dimethoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)-chalcone,
2,6-diethoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)-chalcone,
2,6-di-n-propoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)-chalcone,
2,6-diisopropoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)-chalcone,
2,6-di-n-butoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)-chalcone,
2,6-di-t-butoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)-chalcone,
2,6-dimethoxy-5-propyl-4'-(prop-2-enyloxy)-chalcone,
2,6-diethoxy-5-propyl-4'-(prop-2-enyloxy)-chalcone,
2,6-di-n-propoxy-5-propyl-4'-(prop-2-enyloxy)-chalcone,
2,6-diisopropoxy-5-propyl-4'-(prop-2-enyloxy)-chalcone,
2,6-di-n-butoxy-5-propyl-4'-(prop-2-enyloxy)-chalcone,
2,6-di-t-butoxy-5-propyl-4'-(prop-2-enyloxy)-chalcone,
2,6-dimethoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)-chalcone,

2,6-diethoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)-chalcone,
2,6-di-n-propoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)-chalcone,
2,6-diisopropoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)-chalcone,
2,6-di-n-butoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)-chalcone,
2,6-di-t-butoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)-chalcone,
2,6-dimethoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)-chalcone,
2,6-diethoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)-chalcone,
2,6-di-n-propoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)-chalcone,
2,6-diisopropoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)-chalcone,
2,6-di-n-butoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)-chalcone, or
2,6-di-t-butoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)-chalcone.

72. 2,5-Dimethoxy-4'-prop-2-enyloxychalcone.

73. 2,4-Dimethoxy-4'-hydroxychalcone,
2,4-diethoxy-4'-hydroxychalcone,
2,4-di-n-propoxy-4'-hydroxychalcone,
2,4-diisopropoxy-4'-hydroxychalcone,
2,4-di-n-butoxy-4'-hydroxychalcone,
2,4-di-t-butoxy-4'-hydroxychalcone,
2,4-dimethoxy-5-methyl-4'-hydroxychalcone,
2,4-diethoxy-5-methyl-4'-hydroxychalcone,
2,4-di-n-propoxy-5-methyl-4'-hydroxychalcone,
2,4-diisopropoxy-5-methyl-4'-hydroxychalcone,
2,4-di-n-butoxy-5-methyl-4'-hydroxychalcone,
2,4-di-t-butoxy-5-methyl-4'-hydroxychalcone,
2,4-imethoxy-5-prop-2-enyl-4'-hydroxychalcone,
2,4-diethoxy-5-prop-2-enyl-4'-hydroxychalcone,
2,4-di-n-propoxy-5-prop-2-enyl-4'-hydroxychalcone,
2,4-diisopropoxy-5-prop-2-enyl-4'-hydroxychalcone,
2,4-di-n-butoxy-5-prop-2-enyl-4'-hydroxychalcone, or
2,4-di-t-butoxy-5-prop-2-enyl-4'-hydroxychalcone.

74. A method for the treatment or prophylaxis of a disease caused by a microorganism or a parasite in an animal, including a vertebrate, such as a bird, a fish or a mammal, including a human,

the microorganism or parasite being selected from

parasitic protozoa, in particular tissue and blood protozoa such as *Leishmania*, *Trypanosoma*, *Toxoplasma*, *Plasmodium*, *Pneumocystis*, *Babesia* and *Theileria*; intestinal

protozoan flagellates such as *Trichomonas* and *Giardia;* intestinal protozoan *Coccidia* such as *Eimeria, Isospora, Cryptosporidium; Cappilaria, Microsporidium, Sarcocystis, Trichodina, Trichodinella, Dacthylogurus, Pseudodactylogurus, Acantocephalus, Ichthyophtherius, Botrecephalus;* and intracellular bacteria, in particular *Mycobacterium, Legionella* species, *Listeria,* and *Salmonella,*

which method comprises administering to an animal an effective amount of an aromatic compound, or a prodrug thereof, which aromatic compound contains an alkylating site and which aromatic compound is capable of alkylating the thiol group in N-acetyl-L-cysteine at physiological pH.

75. A method according to claim 74, wherein the aromatic compound or, where appropriate, the prodrug thereof, shows any of the features as defined in any of claims 1-31.

76. A method according to claim 74 or 75, wherein an effective amount of a compound as defined in any of claims 32-73 is administered.

77. A method according to any of claims 74-76, in which the aromatic compound or the prodrug is administered in an amount of about 0.1-80 mg per kg body weight per day, preferably in an amount of about 0.5-20 mg per kg body weight per day.

78. A method according to any of claims 74-77, in which the aromatic compound or the prodrug is administered rectally in the form of suppositories.

79. A method according to any of claims 74-78, in which the aromatic compoundor prodrug is added to animal feed and/or drinking water such as poultry feed and/or drinking water.

80. A method according to any of claims 74-78, wherein the aromatic compound or the prodrug is administered in combination with another antiparasitic, antimycotic, antibiotic or antibabesial drug or anticoccidial agent or another drug against fish parasites.

81. A method according to claim 70, wherein the other antiparasitic drug is an anti-leishmanial drug selected from a pentavalent antimony-sodium gluconate, and allopurinol, or an anitmalarial drug selected from chloroquine and derivatives thereof, quinine, proguanil, cycloguanil, mefloquine, pyrimethamine, and artemisinin.

82. A method according to claim 80, wherein the other antibabesial drug is selected from quinuronium sulfate, pentamidine isethionate, imidocarb or diminazene, or the other anticoccidial drug is selected from sulfonamides, amprocid and coccidiostatic agents selected from ionomycins, such as monensin and salinomycin, or the other drug used against fish

parasites is selected from benzimidazol and formaldehyde, or the additional antibiotic drug is an antituberculous drug selected from isoniazide, ethambutol, pyrazinamid, and rifampicin, or the additional antimycotic drug is selected from amphothericin B, muconarcidol, griseofluvin, and miconazol.

83. A pharmaceutical composition which contains an aromatic compound, or a prodrug thereof, which aromatic compound contains an alkylating site and which aromatic compound is capable of alkylating the thiol group in N-acetyl-L-cysteine at physiological pH, such as a compound as defined in any of claims 32-73 together with another antiparasitic, antimycotic, antibiotic or antibabesial drug or anticoccidial agent or another drug against fish parasites.

84. A pharmaceutical composition according to claim 83, wherein the other antiparasitic drug is an antileishmanial drug selected from a pentavalent antimony-sodium gluconate or allopurinol, or an antimalarial drug selected from chloroquine and derivatives thereof, quinine, proguanil, cycloguanil, mefloquine, pyrimethamine, and artemisinin.

85. A pharmaceutical composition according to claim 83, wherein the other antibabesial drug is selected from quinuronium sulfate, pentamidine isethionate, imidocarb or diminazene, or the other anticoccidial drug is selected from sulfonamides, amprocid and coccidiostatic agents selected from ionomycins, such as monensin and salinomycin, or the other drug used against fish parasites is selected from benzimidazol and formaldehyde, or the additional antibiotic drug is an antituberculous drug selected from isoniazide, ethambutol, pyrazinamid, and rifampicin, or the additional antimycotic drug is selected from amphothericin B, muconarcidol, griseofluvin, and miconazol.

86. A composition which contains an aromatic compound (or a prodrug) as defined in any of claims 32-73 in combination with an animal feed or drinking water for animals, or in combination with at least one pharmaceutical carrier or excipient suitable for administration to an animal, for the treatment or prophylaxis of a disease caused by a microorganism or a parasite selected from

parasitic protozoa, in particular tissue and blood protozoa such as *Leishmania, Trypanosoma, Toxoplasma, Plasmodium, Pneumocystis, Babesia* and *Theileria;* intestinal protozoan flagellates such as *Trichomonas* and *Giardia;* intestinal protozoan *Coccidia* such as *Eimeria, Isospora, Cryptosporidium; Cappilaria, Microsporidium, Sarcocystis; Trichodina, Trichodinella, Dacthylogurus, Pseudodactylogurus, Acantocephalus, Ichthyophtherius, Botrecephalus;* and intracellular bacteria, in particular *Mycobacterium, Legionella* species, *Listeria,* and *Salmonella.*

87. A composition according to claim 86 which is selected from a tablet, a suppository,

and injection fluid.

88. A composition comprising a compound as defined in any of claims 48-73 in combination with an animal feed or drinking water for animals, or in combination with at least one pharmaceutical carrier or excipient.

89. A composition according to claim 86 which is selected from a tablet, a suppository, and injection fluid.

90. A method for controlling transmission of parasitic diseases caused by parasites which have part of their life cycle in a vector, said method comprising applying an aromatic compounds, or a prodrug thereof, which aromatic compound contains an alkylating site and is capable of alkylating the thiol group in N-acetyl-L-cysteine at physiological pH, preferably a compound as defined in any of claims 32-73, to a locus which is a habitat of the vector so as to eradicate the parasites.

91. The method according to claim 90, wherein the application is performed by spraying a sprayable composition containing the aromatic compound.

92. A compound of the general formula I as defined in any of claims 32-36 with the proviso that W is a group -C≡C-.

93. A method for the preparation of a compound of the general formula I as defined in claim 32, said method comprising

a) for the preparation of a compound of the general formula I, in which both R are H, reacting a ketone of the general formula I'

$$X\text{-}Ar^1\text{-}CO\text{-}CH_3 \qquad I'$$

wherein X and Ar^1 are as defined in claim 32,
with an aldehyde of the general formula I''

$$HCO\text{-}Ar^2\text{-}Y \qquad I''$$

wherein Ar^2 and Y are as defined in claim 32, or

b) for the preparation of a compound of the general formula I in which W is -C≡C-, reacting an activated derivative of a carboxylic acid of the general formula II'

$$X\text{-}Ar^1\text{-}COOH \qquad II'$$

wherein X and Ar^1 are as defined in claim 32,

with an ethyne derivative of the general formula II''

$$H\text{-}C{\equiv}C\text{-}Ar^2\text{-}Y \qquad \text{II''}$$

wherein Ar^2 and Y are as defined in claim 32, or

c) for the preparation of a compound of the general formula I, in which W is -CR=CR-, wherein R is as defined in claim 32, dehydrating a β-hydroxyketone of the general formula E,

$$X\text{-}Ar^1\text{-}CO\text{-}CRH\text{-}C(OH)R\text{-}Ar^2\text{-}Y \qquad E$$

wherein X, Y, Ar^1, Ar^2 and R are as defined in claim 32, or

d) for the preparation of a compound of the general formula I, wherein W is -C≡C-, eliminating HLea from a ketone of the general formula E1,

$$X\text{-}Ar^1\text{-}CO\text{-}CH{=}C(Lea)\text{-}Ar^2\text{-}Y \qquad E1$$

wherein X, Y, Ar^1 and Ar^2 are as defined in claim 32, and Lea is a halide or another leaving group such as hydroxy, alkoxy, tosyloxy, or trifluoromethanesulfonoxy, or

e) for the preparation of a compound of the general formula I, wherein W is -CR=CH-, wherein R is as defined in claim 32, reacting an aldehyde or ketone of the general formula F

$$Y\text{-}Ar^2\text{-}CRO \qquad F$$

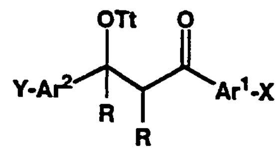
in which Y and Ar^2 are as defined in claim 32, with a phosphorus ylide (also called a phosphorane) of the general formula G,

$$T_3\text{-}P{=}CR\text{-}CO\text{-}Ar^1\text{-}X \qquad G$$

in which T is an aliphatic, alicyclic or aromatic group, and Ar^1, X and R are as defined in claim 32, or

f) for the preparation of a compound of the general formula I, in which W is -CR=CR- in which R is as defined in claim 32, eliminating HOTt from a ketone of the

general formula K



K

wherein X, Y, Ar^1, Ar^2 and R are as defined in claim 32, and Tt is hydrogen, alkyl, tosyl, trifluoromethanesulfonyl or acyl, or

g) for the preparation of a compound of the general formula I, in which W is -CR=CR- in which R is as defined in claim 32, reacting a cinnamic acid of the general formula L

Y-Ar^2-CR=CR-COQ L

wherein Y, Ar^2 and R are as defined in claim 32, and Q is a hydroxy group, a carboxylate or a halogen atom, with an aromate of the general formula M

X-Ar^1 M

wherein X and Ar^1 are as defined in claim 32.

94. A method according to claim 93 c), in which the β-hydroxyketone of the general formula E is prepared by reacting an aldehyde of the general formula A

X-Ar^1-CHO A

in which Ar^1 and X are as defined in claim 32, with hydroxylamine or a salt thereof to form the corresponding oxime of the general formula B

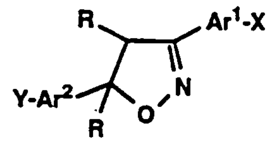
X-Ar^1-CH=N-OH B

reacting the oxime of the general formula B with a halogenating agent, eliminating hydrogen halide, and subsequently adding an olefin of the general formula C

CHR=CR-Ar^2-Y C

in which Ar^2, Y and R are as defined in claim 32, to form the corresponding isoxazoline of

the formula D



D

reducing the isoxazoline of the formula D and hydrolyzing the reduction product to form a β-hydroxyketone of the general formula E.

95. A method according to claim 93 d), in which the β-hydroxyketone of the general formula E1 is prepared by reacting an aldehyde of the general formula A

$X\text{-}Ar^1\text{-}CHO$ A

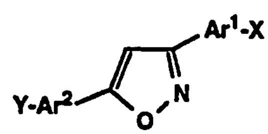
in which Ar^1 and X are as defined in claim 32, with hydroxylamine or a salt thereof to form the corresponding oxime of the general formula B

$X\text{-}Ar^1\text{-}CH{=}N\text{-}OH$ B

reacting the oxime of the general formula B with a halogenating agent and subsequently adding an acetylene of the general formula C1

$CH{\equiv}C\text{-}Ar^2\text{-}Y$ C1

in which Ar^2, Y and R are as defined in claim 32, to form the corresponding isoxazole of the formula D1



D1

reducing the isoxazole of the formula D1 and hydrolysing the reduction product.

96. A method according to claim 93 c) or d) wherein the β-hydroxyketone of the general formula E or E1 is prepared *in situ* by reduction and hydrolysis of the isoxazoline D or isoxazole D1 in one step.

97. A method according to claim 93 f) wherein the ketone of the general formula K in which Tt is hydrogen, is prepared by reacting a compound of the general formula H

$$X\text{-}Ar^1\text{-}CV{=}CH_2 \qquad\qquad H$$

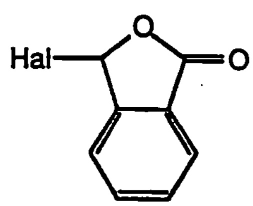
in which X and Ar^1 are as defined in claim 32, and in which V represents a secondary amine group, such as a morpholino or piperidino group, with an aldehyde of the general formula J

$$Y\text{-}Ar^2\text{-}CHO \qquad\qquad J$$

in which Y and Ar^2 are as defined in claim 32, to form an intermediate which is hydrolyzed to reconstitute the carbonyl function.

98. A method for preparing a compound of the general formula I as defined in claim 32, wherein Z is a group D or E as defined in claim 33, comprising reacting the corresponding compound of the general formula I wherein X and/or Y is AZ wherein A is as defined in claim 32, in particular -O- or -NH-, and Z is H, with the appropriate halide of the general fomula D-Hal or E-Hal

$$Hal\text{-}CR^*R^{**}\text{-}O\text{-}CO\text{-}R''' \qquad\qquad D\text{-}Hal$$



E-Hal

in which R^*, R^{**} and R''' are as defined in claim 33, and Hal is a halogen atom such as chlorine, bromine or iodine.

99. A method according to claim 98, wherein the halide D-Hal is iodomethyl pivaloylate.

100. A method for preparing a compound of the general formula I as defined in claim 32, wherein Z is a carboxylic acid residue (A) as defined in claim 33, comprising reacting the corresponding compound of the general formula I wherein X and/or Y is AZ, wherein A is as defined in claim 32, and Z is H, with a reactive derivative of the carboxylic acid HO-CO-R', wherein R' is as defined in claim 33, the reactive acid derivate being, in particular, selected from activated esters, anhydrides, and acid halides, such as the acid chloride.

101. A method for preparing a compound of the general formula I as defined in claim 32,

wherein Z is a dimethylcarbamoyl group, comprising reacting the corresponding compound of the general formula I, in which X and/or Y is AZ, wherein A is as defined in claim 32, and Z is H, with an activated derivative of N,N-dimethylcarbamic acid.

102. A method for preparing a compound of the general formula I as defined in claim 32, wherein Z is an alkoxyalkyl group (C) as defined in claim 33, comprising reacting the corresponding compound of the general formula I, in which X and/or Y is AZ, wherein A is as defined in claim 32, and Z is H, with an alkyl-α-haloalkyl ether.

103. A method for preparing a compound of the general formula I as defined in claim 32, wherein R is halogen comprising, reacting the corresponding compound of the general formula I in which W is -CH=CH- with bromine followed by dehydrobromination of the formed dibromide using potassium acetate in methanol.

1/15

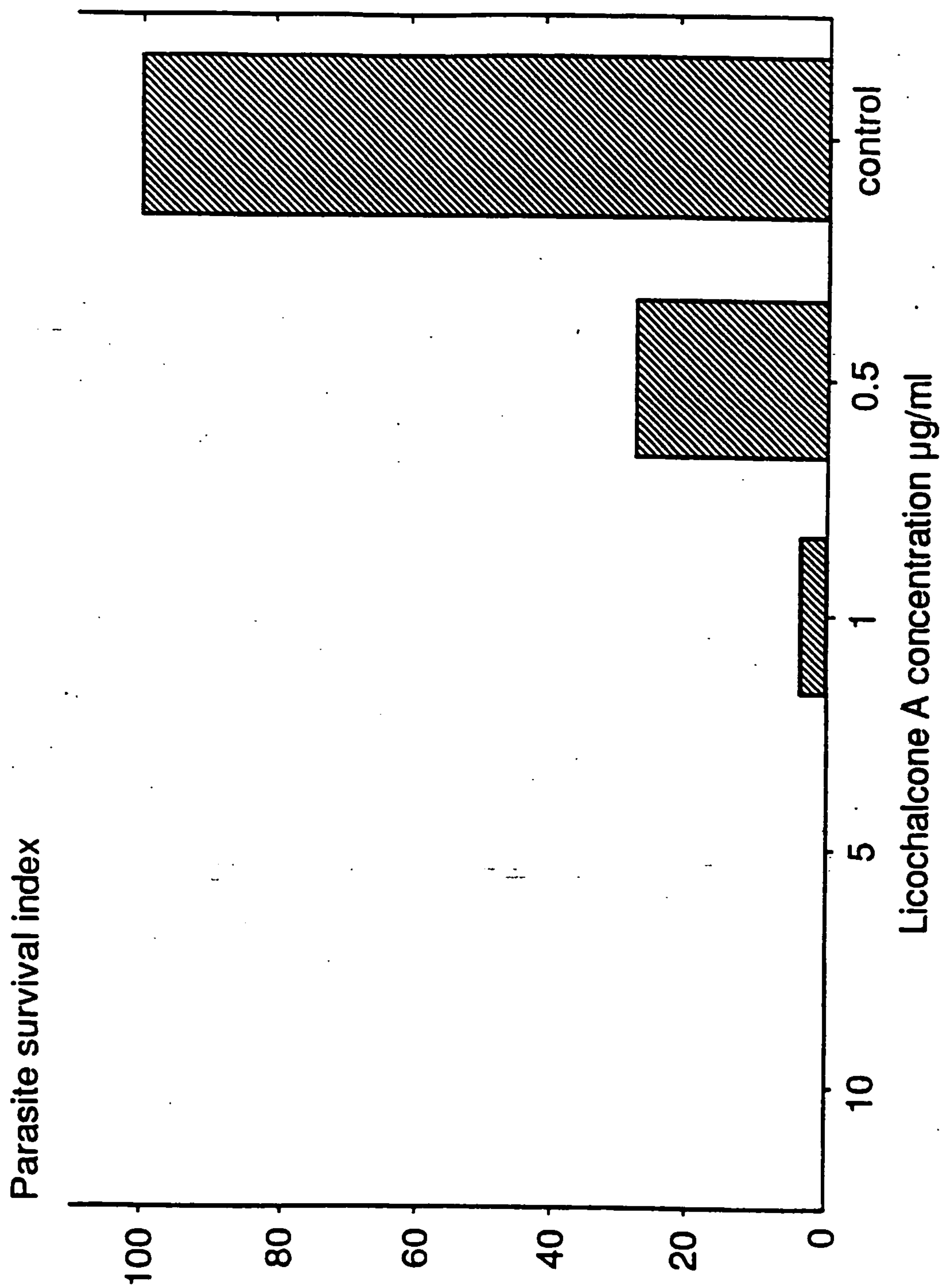
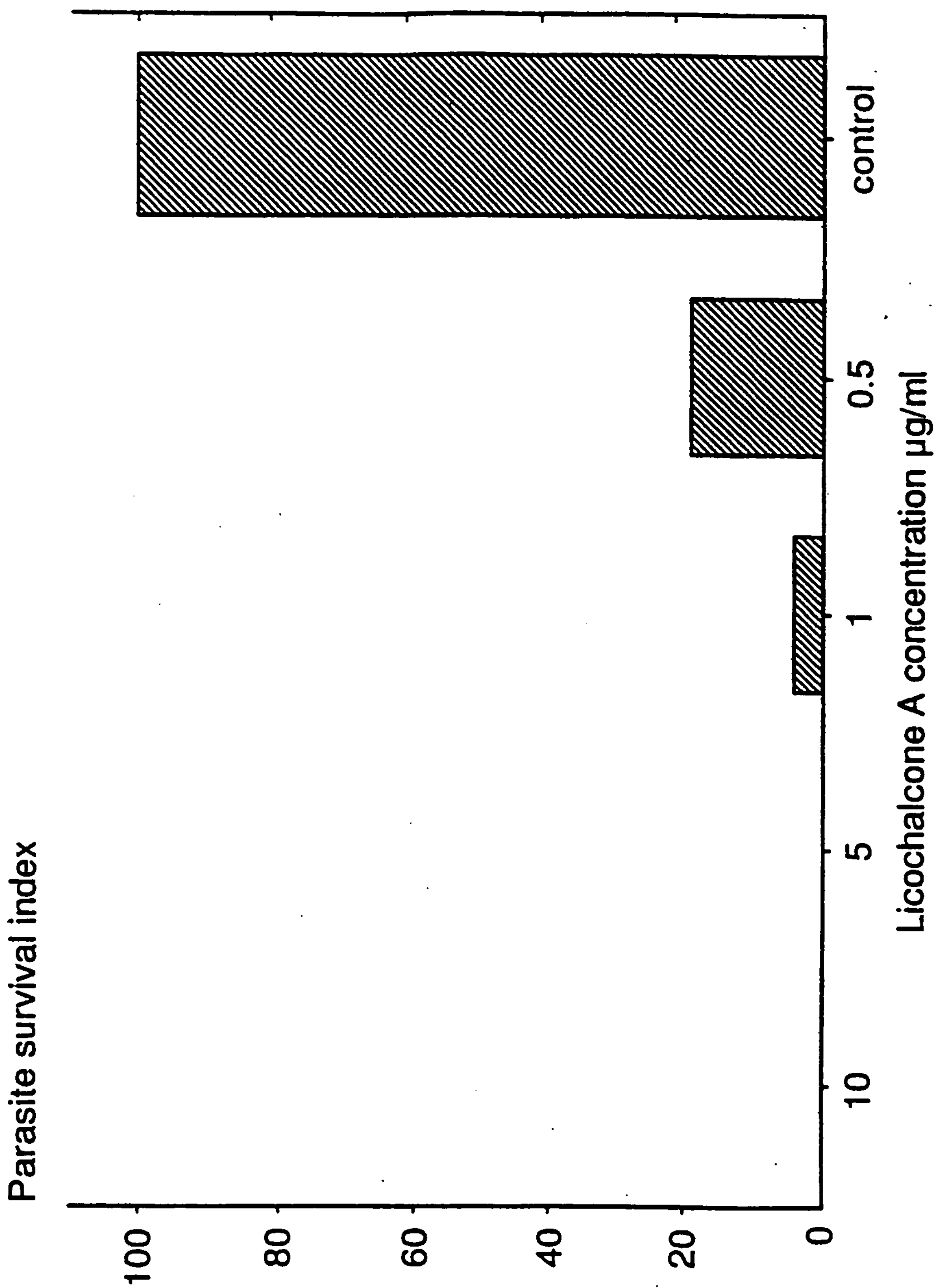

Parasite survival index
100
80
60
40
20
0
control
0.5
1
5
10
Licochalcone A concentration µg/ml

Fig. 1



Parasite survival index
100
80
60
40
20
0
control
0.5
1
5
10
Licochalcone A concentration µg/ml

Fig. 2

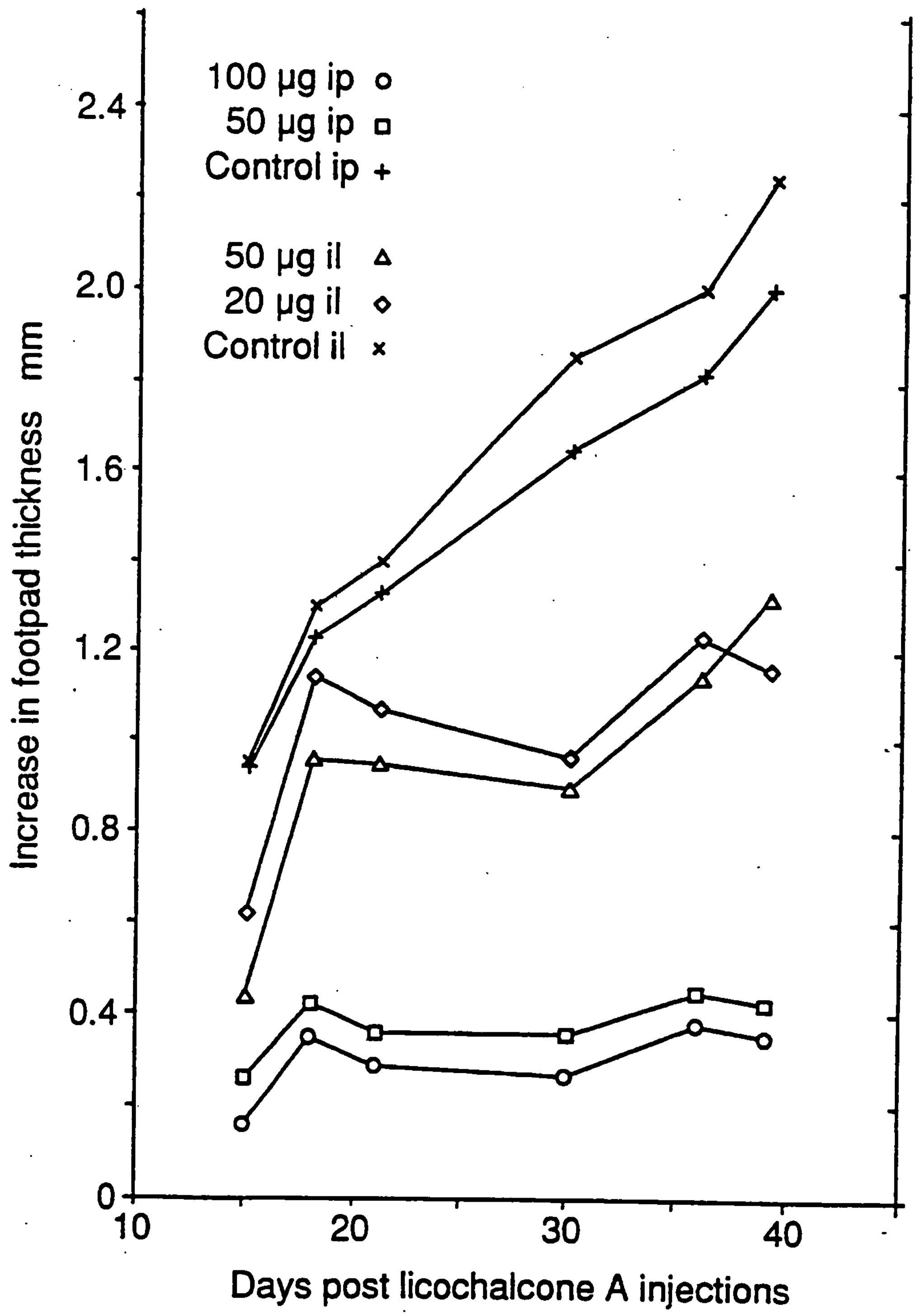

100 µg ip
50 µg ip
Control ip
50 µg il
20 µg il
Control il
Increase in footpad thickness mm
2.4
2.0
1.6
1.2
0.8
0.4
0
10
20
30
40
Days post licochalcone A injections

Fig. 3

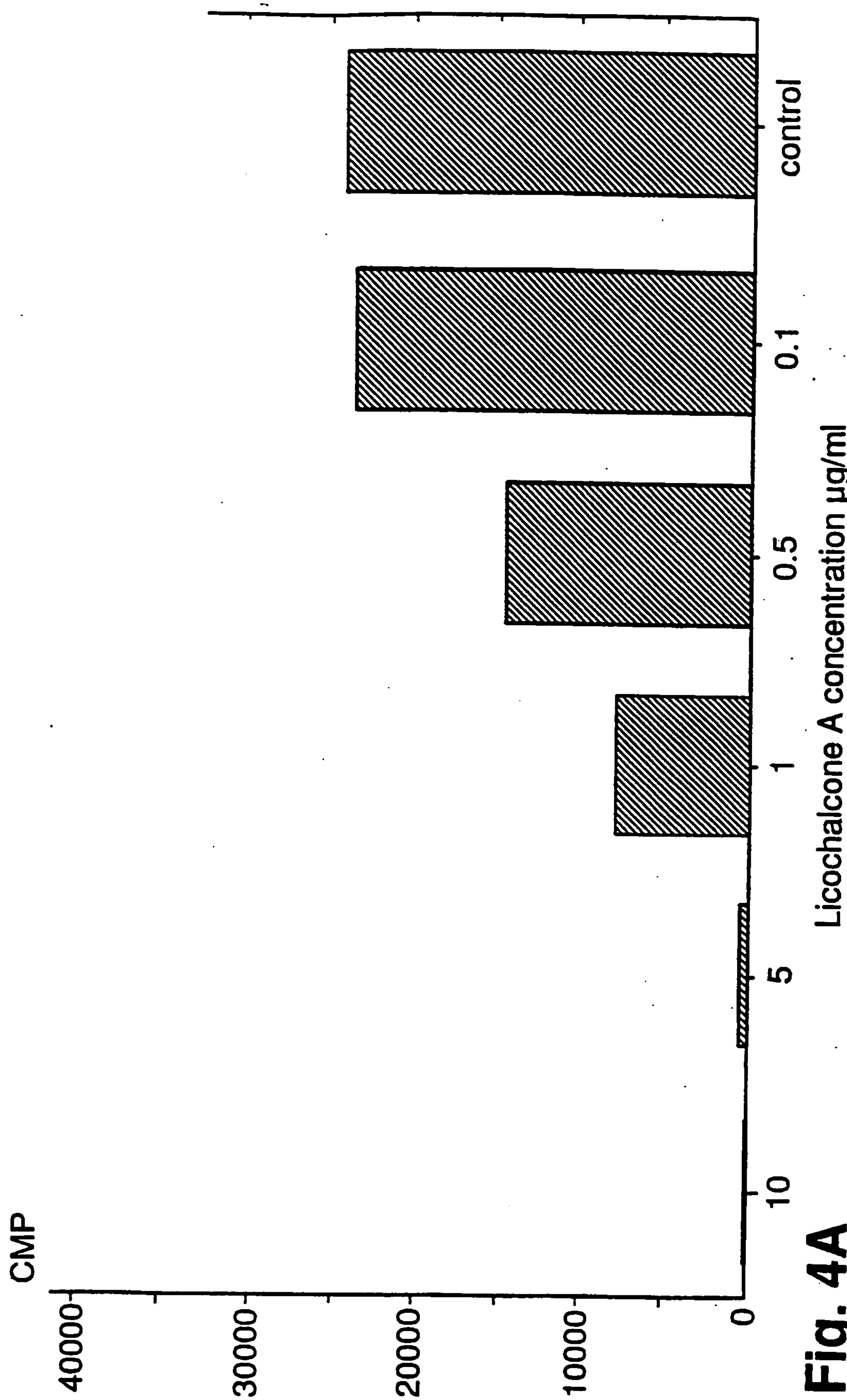

CMP
40000
30000
20000
10000
0
control
0.1
0.5
1
5
10
Licochalcone A concentration µg/ml

Fig. 4A

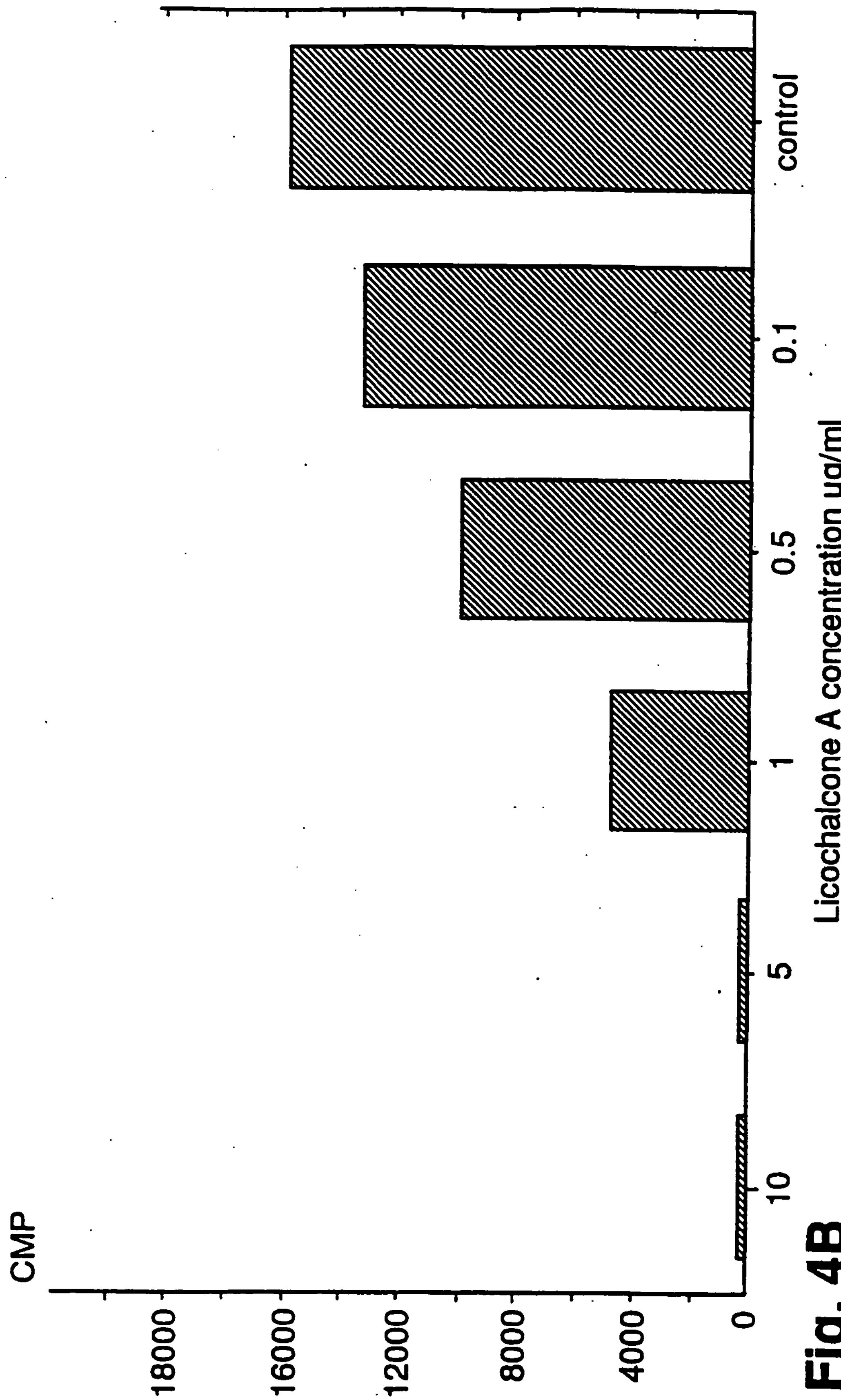

CMP
18000
16000
12000
8000
4000
0
control
0.1
0.5
1
5
10
Licochalcone A concentration µg/ml

Fig. 4B

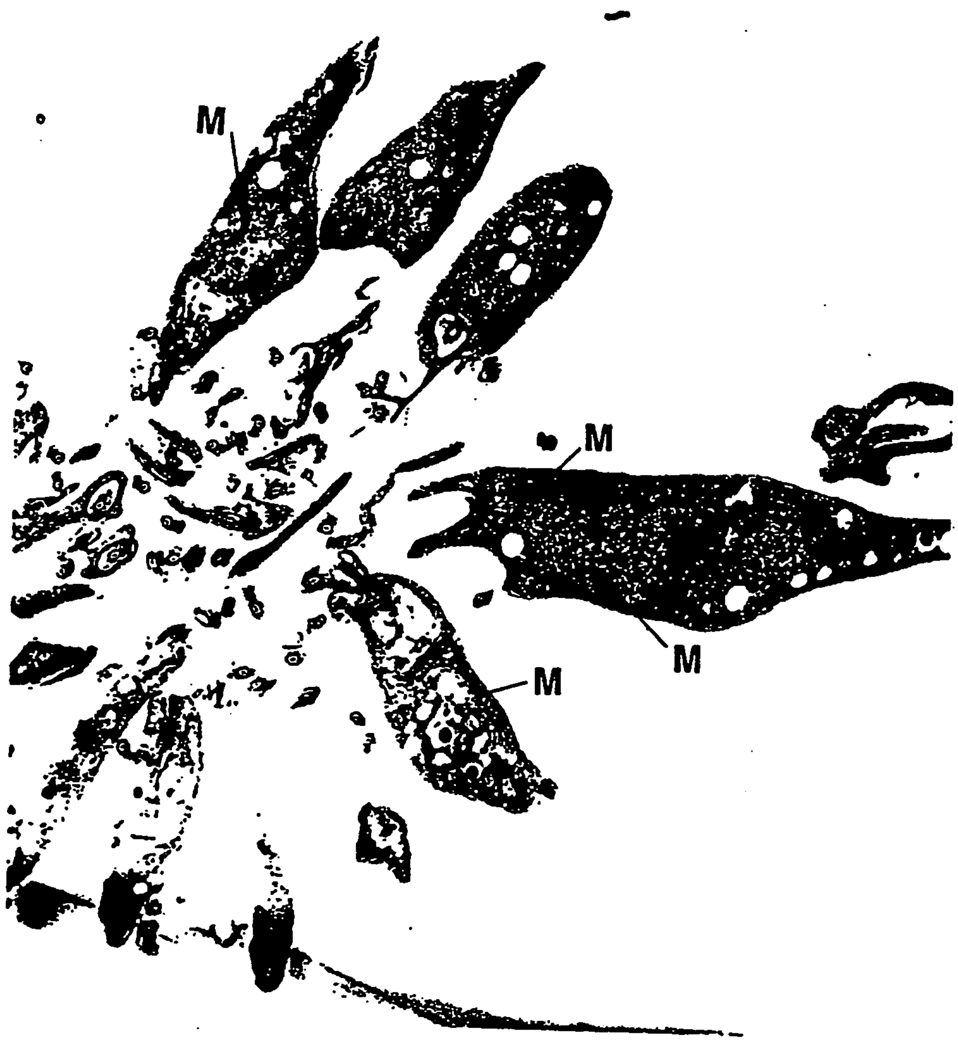


Fig. 5

Fig. 6

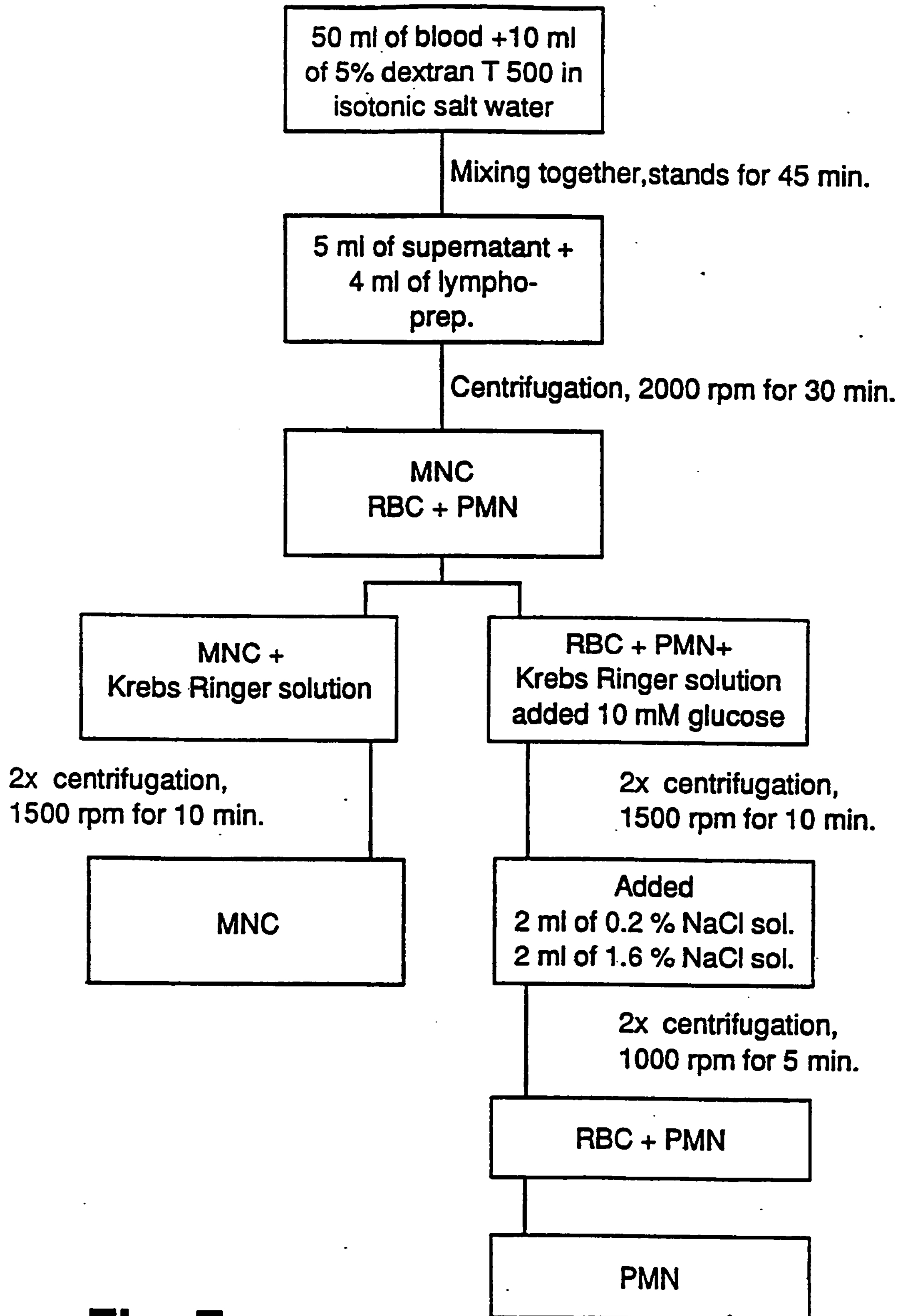

50 ml of blood +10 ml of 5% dextran T 500 in isotonic salt water
Mixing together,stands for 45 min.
5 ml of supernatant + 4 ml of lympho-prep.
Centrifugation, 2000 rpm for 30 min.
MNC
RBC + PMN
MNC + Krebs Ringer solution
RBC + PMN+ Krebs Ringer solution added 10 mM glucose
2x centrifugation, 1500 rpm for 10 min.
2x centrifugation, 1500 rpm for 10 min.
MNC
Added
2 ml of 0.2 % NaCl sol.
2 ml of 1.6 % NaCl sol.
2x centrifugation, 1000 rpm for 5 min.
RBC + PMN
PMN

Fig. 7

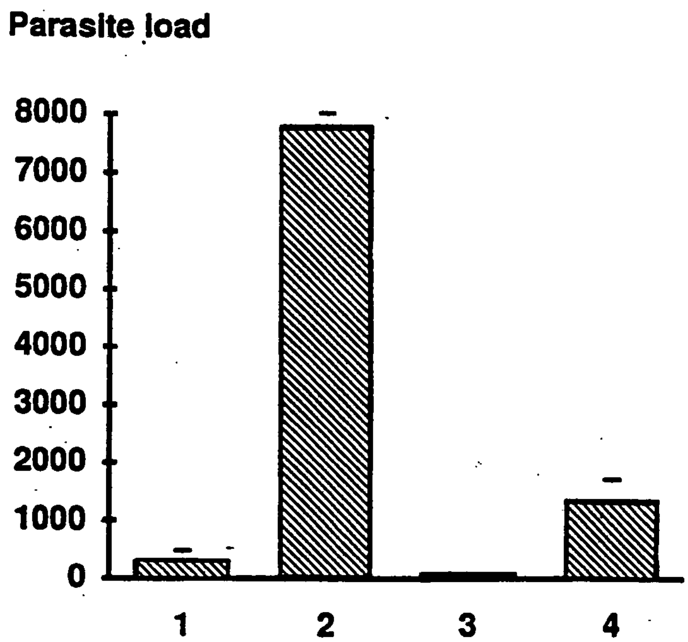


Fig. 8

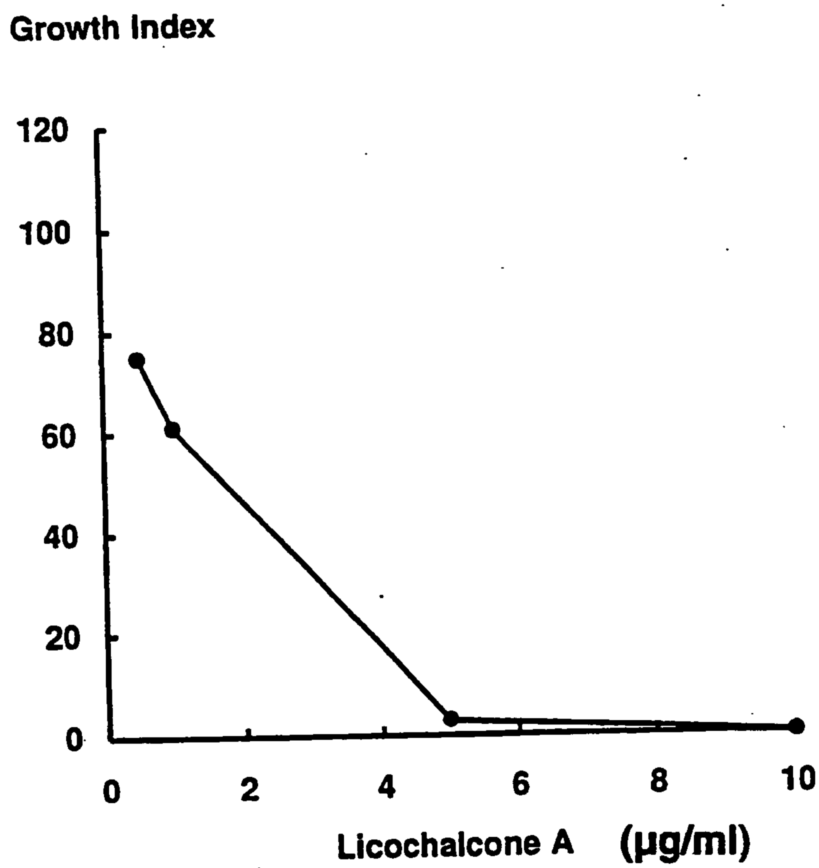

Growth Index
120
100
80
60
40
20
0
0
2
4
6
8
10
Licochalcone A (µg/ml)

Fig. 9A

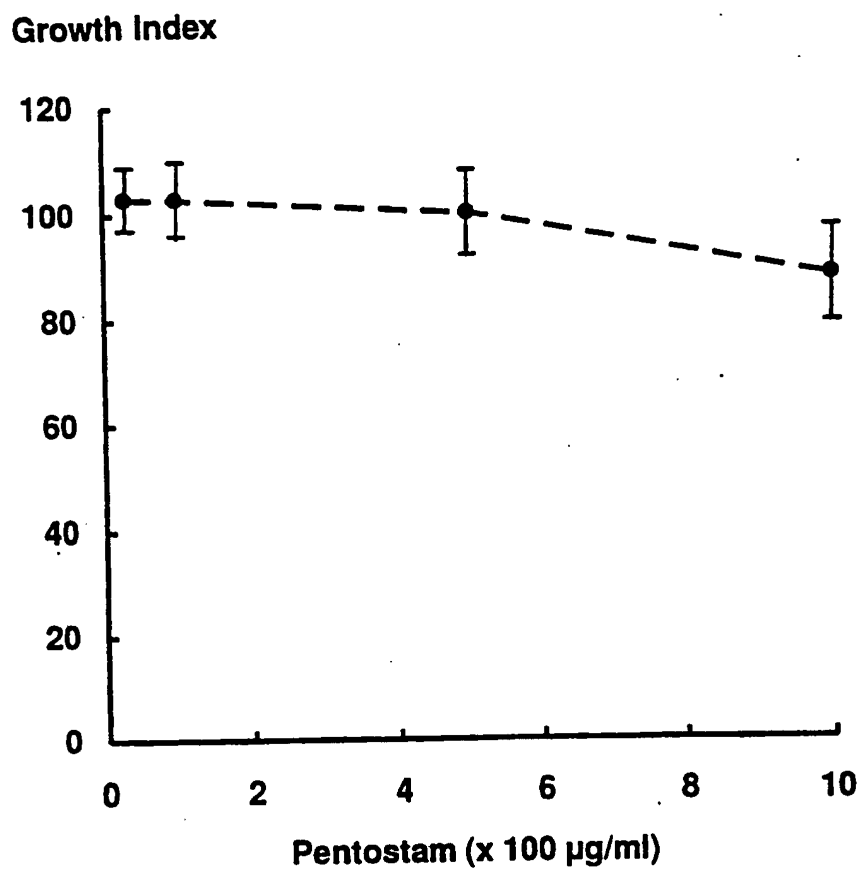

Growth Index
120
100
80
60
40
20
0
0
2
4
6
8
10
Pentostam (x 100 µg/ml)

Fig. 9B

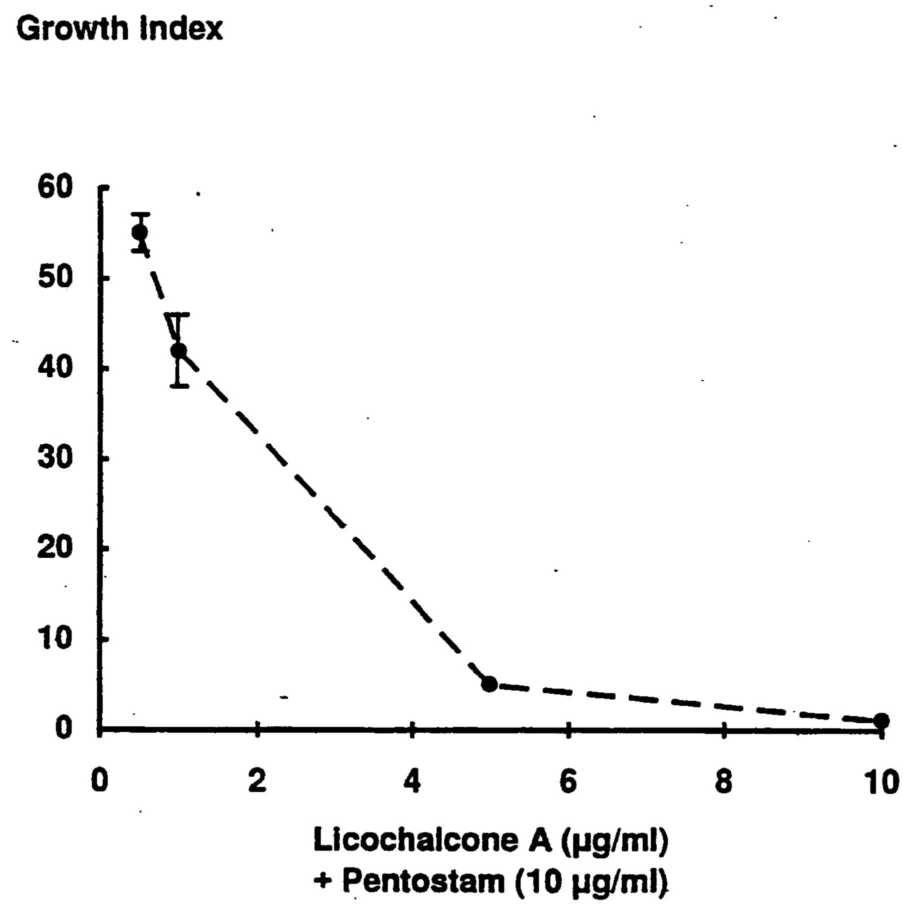

Growth Index
60
50
40
30
20
10
0
0
2
4
6
8
10
Licochalcone A (µg/ml)
+ Pentostam (10 µg/ml)

Fig. 9C

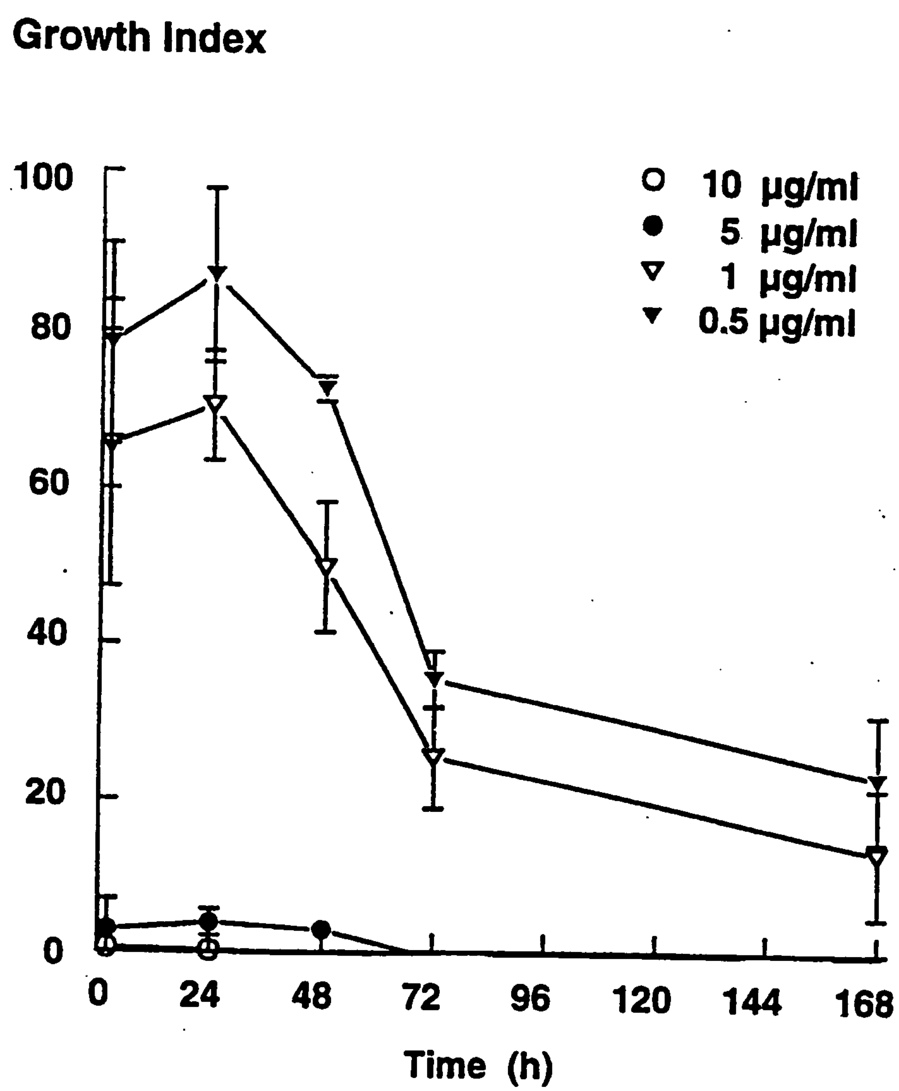

Growth Index
100
80
60
40
20
0
10 µg/ml
5 µg/ml
1 µg/ml
0.5 µg/ml
0
24
48
72
96
120
144
168
Time (h)

Fig. 10A

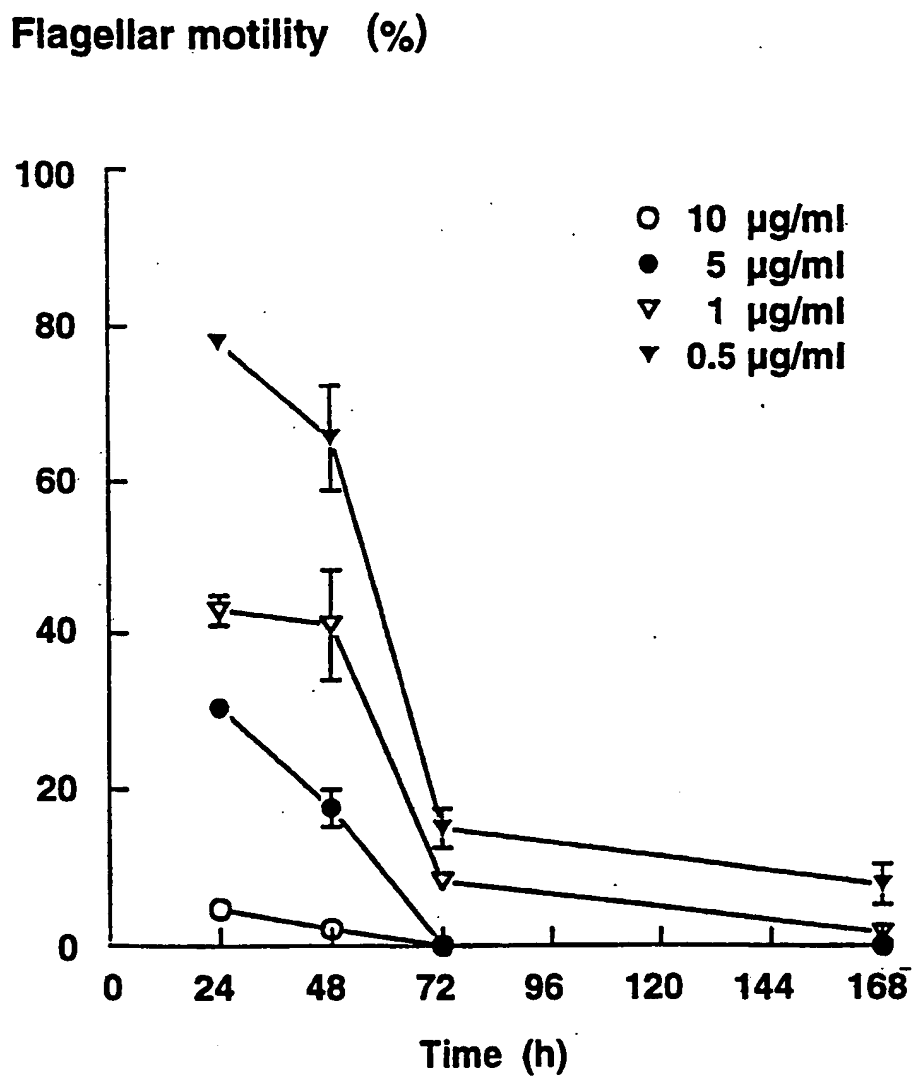

Flagellar motility (%)
100
80
60
40
20
0
10 µg/ml
5 µg/ml
1 µg/ml
0.5 µg/ml
0
24
48
72
96
120
144
168
Time (h)

Fig. 10B

Fig. 11A

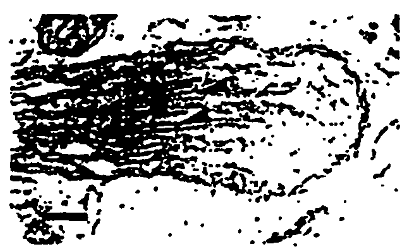


Fig. 11B

Fig. 11C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 93/00088

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: A61K 31/12, A61K 31/135, C07C 49/84, C07C 69/007, C07C 225/22, C07C 323/22

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: A61K, C07C, C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CA, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	STN International, File Biosis, STN accession no. 87:186434, Pappano N B et al: "Relation of the carbonyl region with the bacteriostatic activity of chalcones", & Comun Biol S (2) 1986, p. 179-192 (RECD. 1987) --	1-66,76-82, 85
X	STN International, File Medline, Medline accession no. 77143880,Hall I H et al: "Antitumor agents. 21. A proposed mechanism for inhibition of cancer gowth by tenulin and helenalin and related cyclopentenones", & J Med Chem. (1977 Mar) 20 (3) 333-7 --	1-47,76-82, 85

[X] Further documents are listed in the continuation of Box C.   [X] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" erlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 June 1993	11 -06- 1993
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Gerd Wranne Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 93/00088

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Pharmazie, Volume 44, 1989, M. Szajda et al, "New alkoxycarbonylalkyloxychalcones and their alfa, beta-dibromo derivatives of potential antimicrobial activity", see pages 190-191 --	1-66,76-82
X	Patent Abstracts of Japan, Vol 12,No 33, C-472, abstract of JP, A, 62-181202 (HIGASHIMARU SHOYU K.K. et al), 8 August 1987 (08.08.87) --	1-66,76-82, 85
X	Chemical Abstracts, Volume 115, No 5, 5 August 1991 (05.08.91), (Columbus, Ohio, USA), page 92, THE ABSTRACT No 41976j, JP, A, 2304024, (MINOPHAGEN PHARMACEUTICAL CO., LTD.) 17 December 1990 (17.12.90) --	1-66,76-82, 85
X	EP, A1, 0013960 (F. HOFFMANN-LA ROCHE & CO. AG), 6 August 1980 (06.08.80), see the claims --	1-66,76-82, 85-97
X	US, A, 4867964 (SERGE FORESTIER ET AL), 19 Sept 1989 (19.09.89), see claim 1 --	48-66,85
X	EP, A1, 0328669 (NIPPON OIL AND FATS COMPANY, LIMITED), 23 August 1989 (23.08.89), see page 2, line 9 - line 31; the claims --	1-66,76-82, 85-97
A	US, A, 4279930 (C.M. HALL ET AL), 21 July 1981 (21.07.81) --	1-66,76-82, 85

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 93/00088

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 67-75,83,84
because they relate to subject matter not required to be searched by this Authority, namely:
See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [X] Claims Nos.: 1-66,76-82,85-97 searched incompletely
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
The formulation of the claims is so complicated, because of the distinct combinations of the meanings of the variable parts that it does not comply with art. 6 PCT prescribing that the claims shall be clear and concise. For these reasons the search has been incomplete and has essentially been limited to the examples.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

30/04/93

International application No.
PCT/DK 93/00088

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
JP-A-	2304024	17/12/90	NONE		
EP-A1-	0013960	06/08/80	SE-T3-	0013960	
			AT-T-	1744	15/11/82
			AU-B-	537855	19/07/84
			AU-A-	5474480	31/07/80
			CA-A-	1124254	25/05/82
			JP-A-	56007736	27/01/81
			US-A-	4605674	12/08/86
US-A-	4867964	19/09/89	BE-A-	1000652	28/02/89
			CA-A-	1299581	28/04/92
			CH-A-	674933	15/08/90
			DE-A-	3742690	30/06/88
			FR-A,B-	2608150	17/06/88
			GB-A,B-	2198945	29/06/88
			JP-A-	63165311	08/07/88
			LU-A-	86715	14/07/88
EP-A1-	0328669	23/08/89	WO-A-	8900989	09/02/89
US-A-	4279930	21/07/81	NONE		